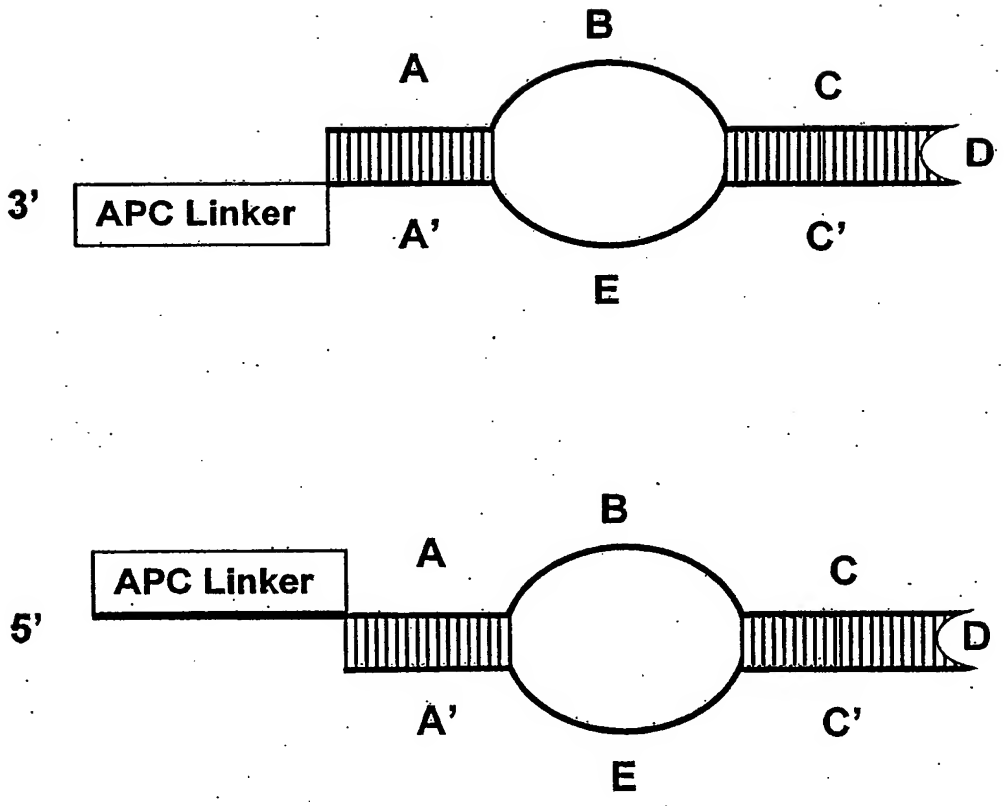


FIGURE 1

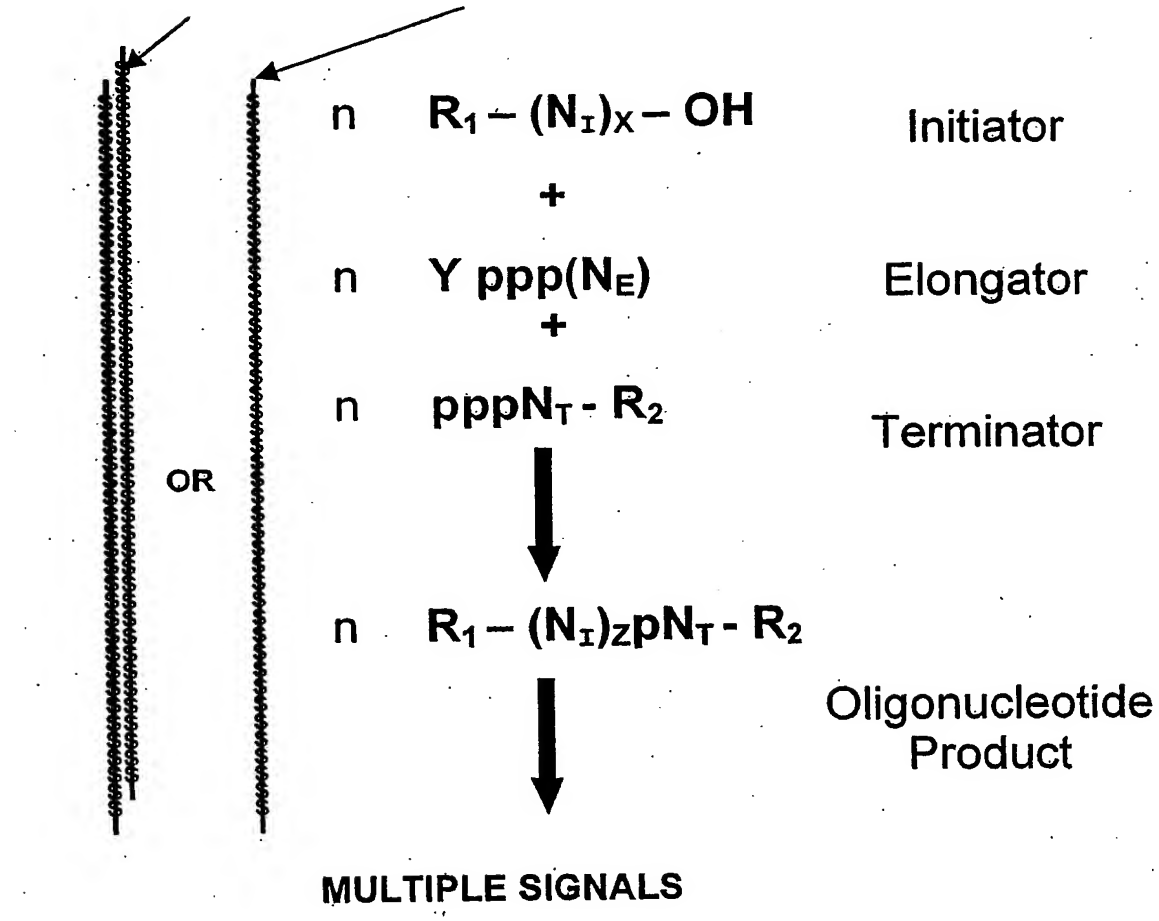

Double Stranded or Single Stranded DNA or RNA
OR
n $R_1 - (N_I)_X - OH$
Initiator
+
n Y ppp(N_E)
Elongator
+
n $pppN_T - R_2$
Terminator
n $R_1 - (N_I)_Z pN_T - R_2$
Oligonucleotide Product
MULTIPLE SIGNALS

FIGURE 2

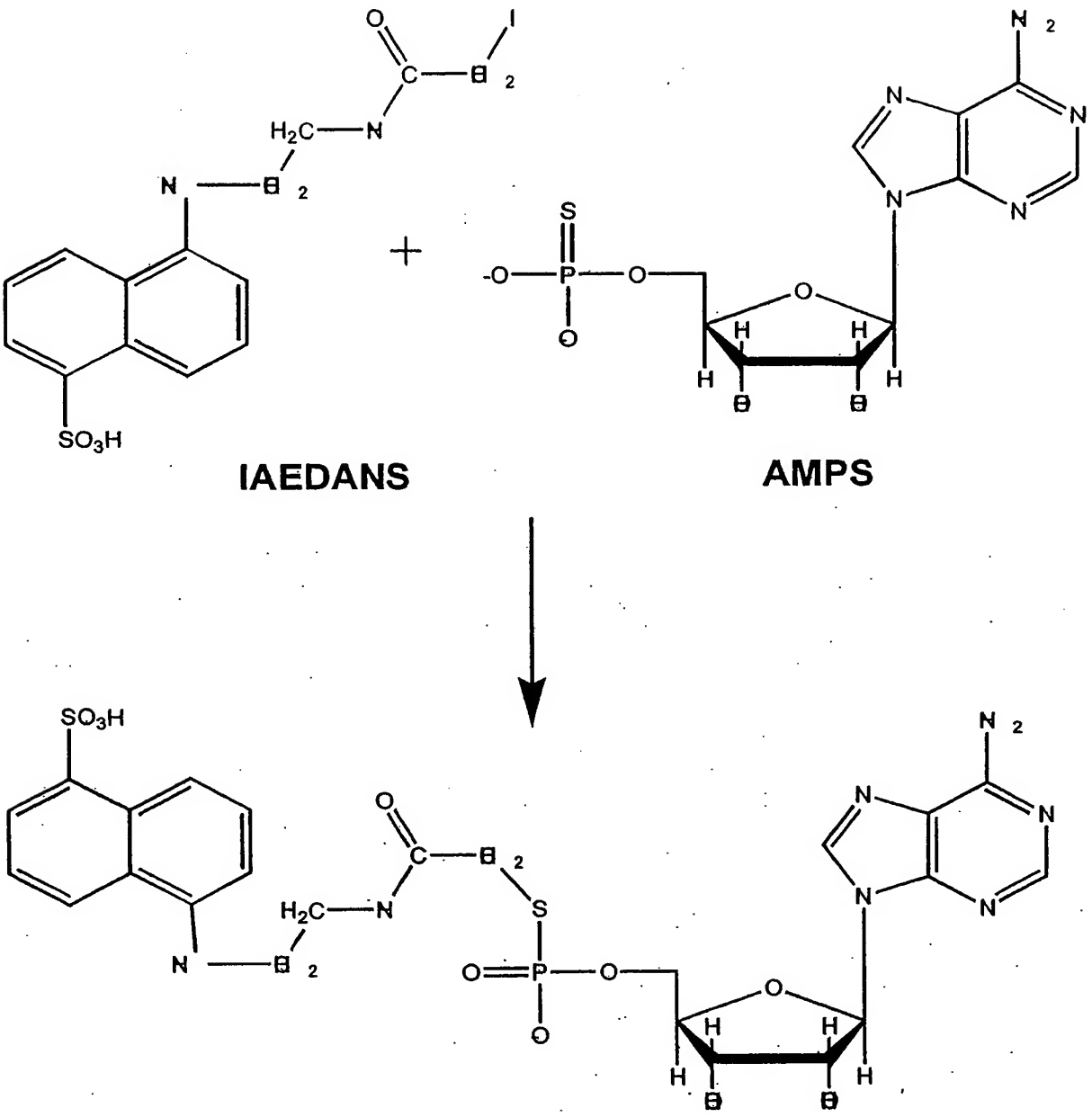
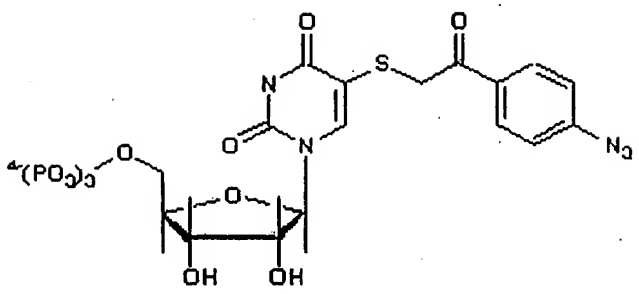
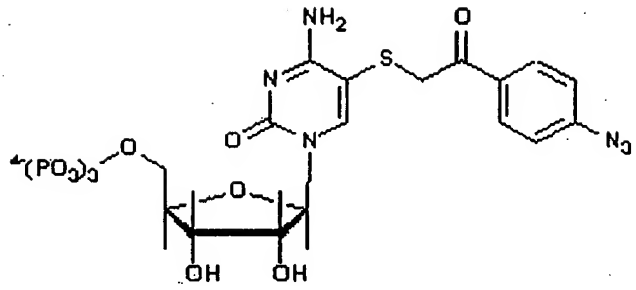


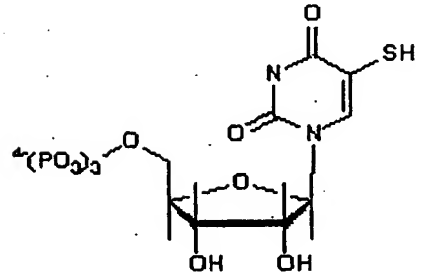
FIGURE 3



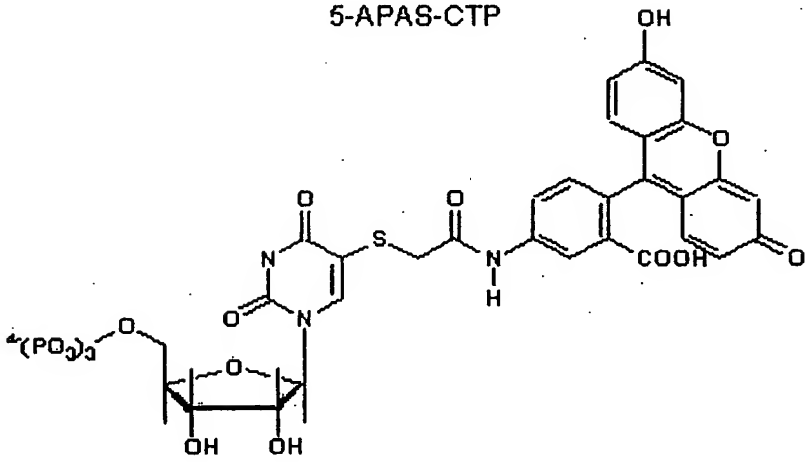
5-APAS-UTP



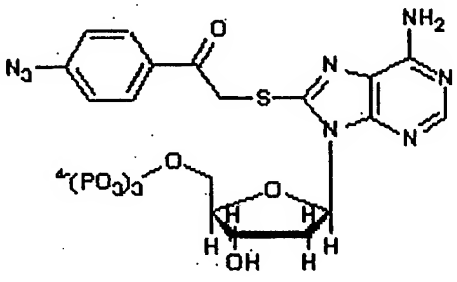
5-APAS-CTP



5-SH-UTP

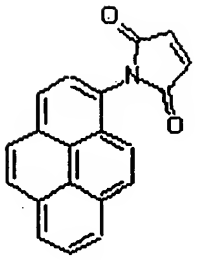


5-SF-UTP

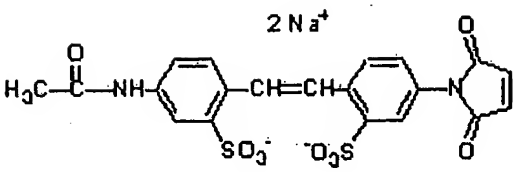


8-APAS-ATP

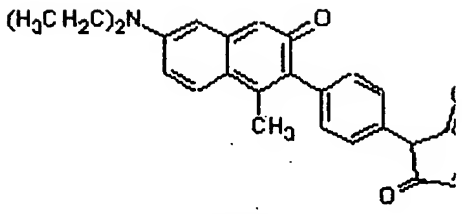
FIGURE 4



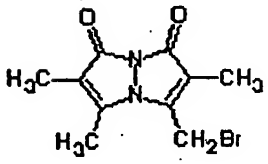
pyrene



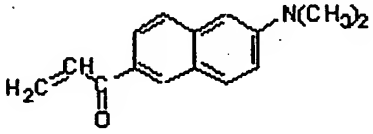
stilbene



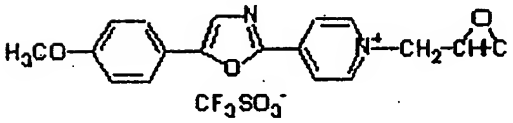
coumarine



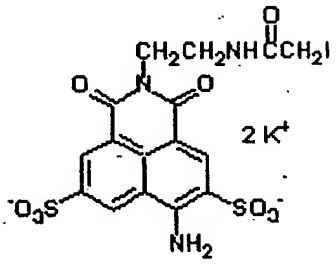
bimane



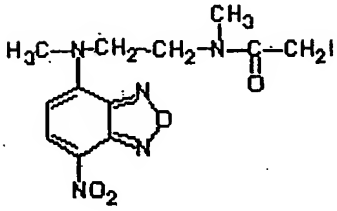
naphthalene



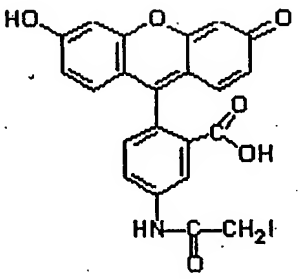
pyridyloxazole



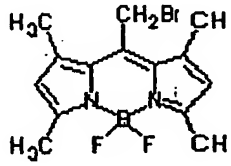
naphthalimide



NBD



fluorescein



BODIPY™

FIGURE 5

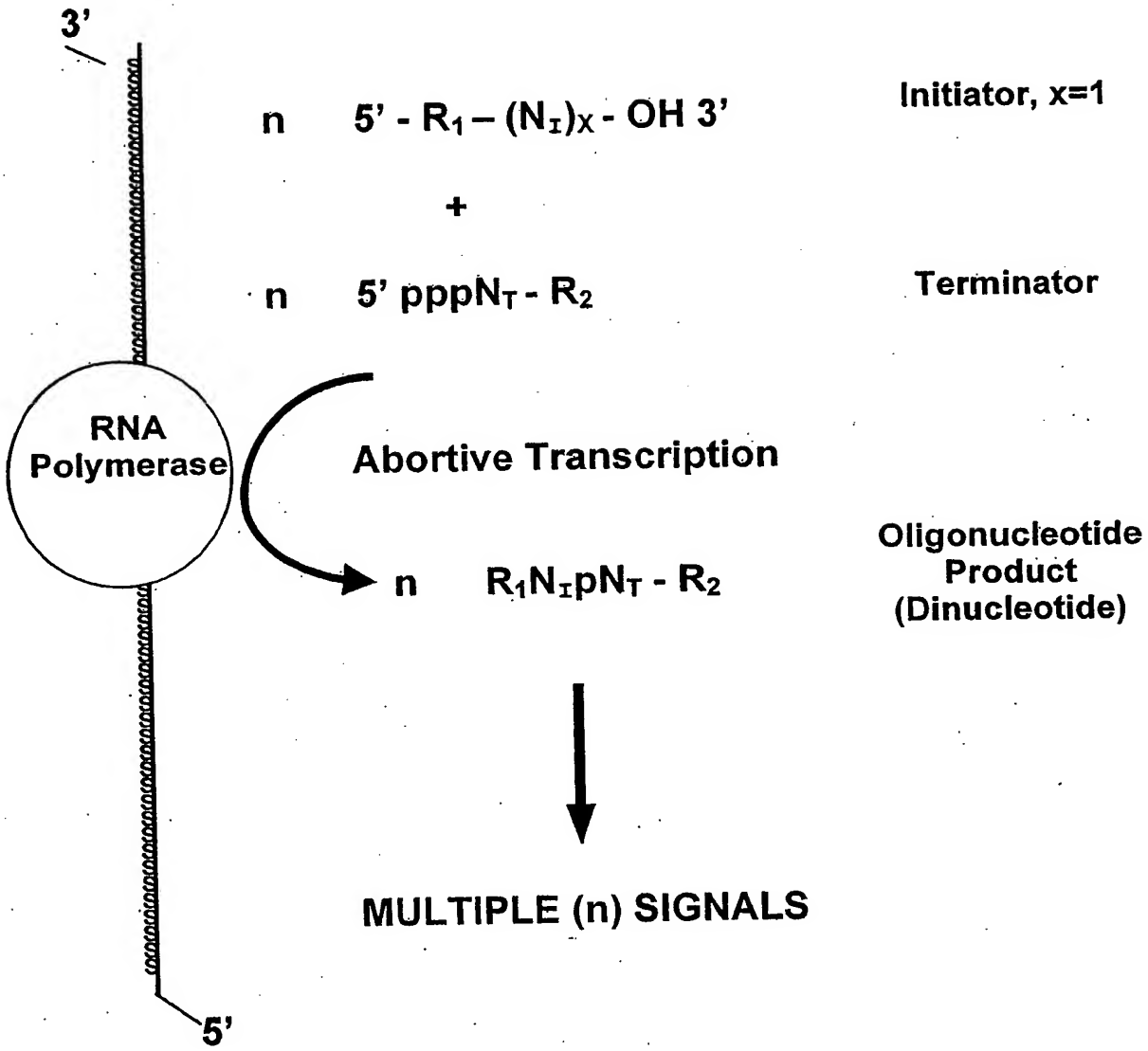

3'
n 5' - R1 – (NI)X - OH 3'
Initiator, x=1
+
n 5' pppNT - R2
Terminator
RNA Polymerase
Abortive Transcription
n R1NIpNT - R2
Oligonucleotide Product (Dinucleotide)
MULTIPLE (n) SIGNALS
5'

FIGURE 6

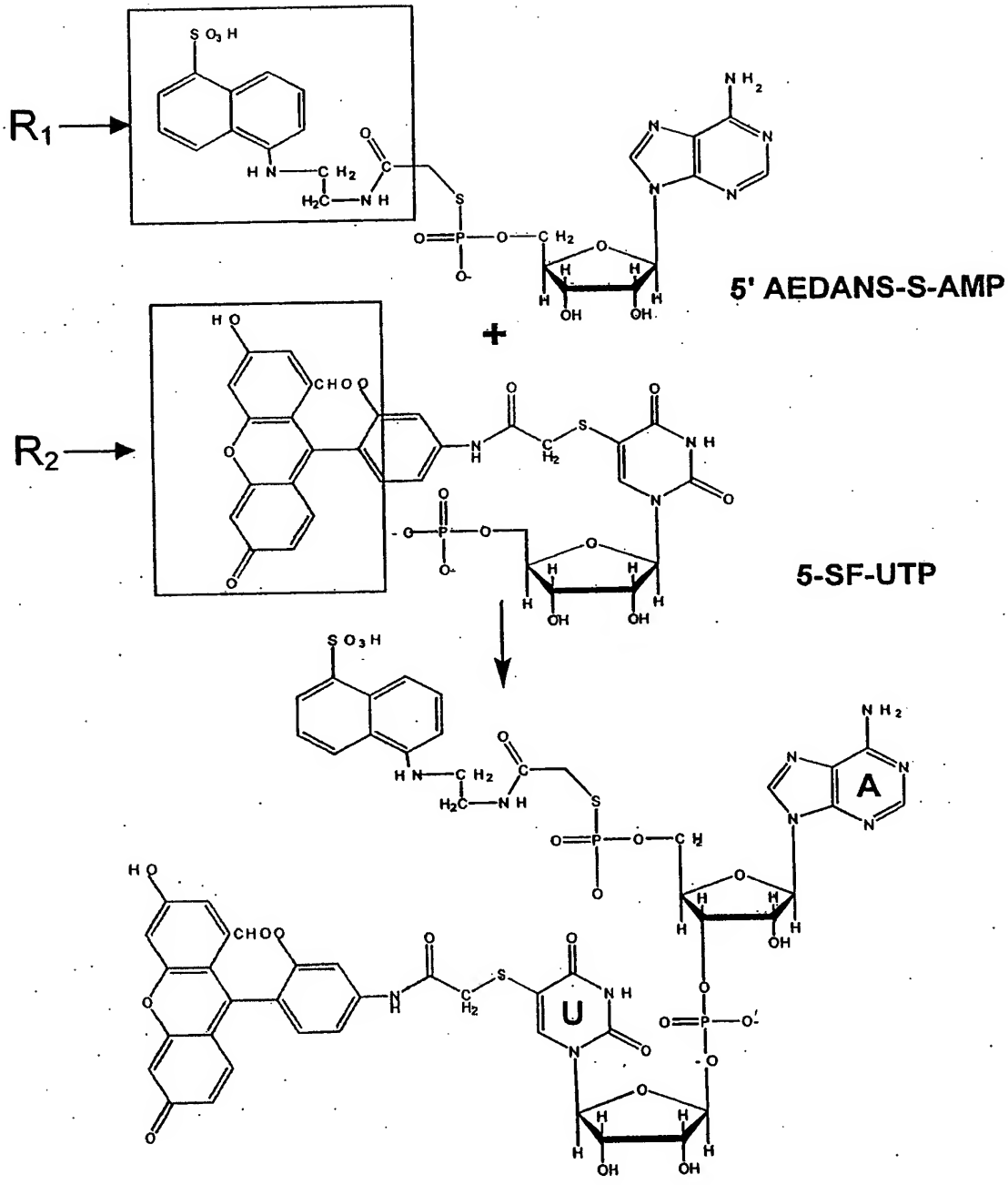


FIGURE 7

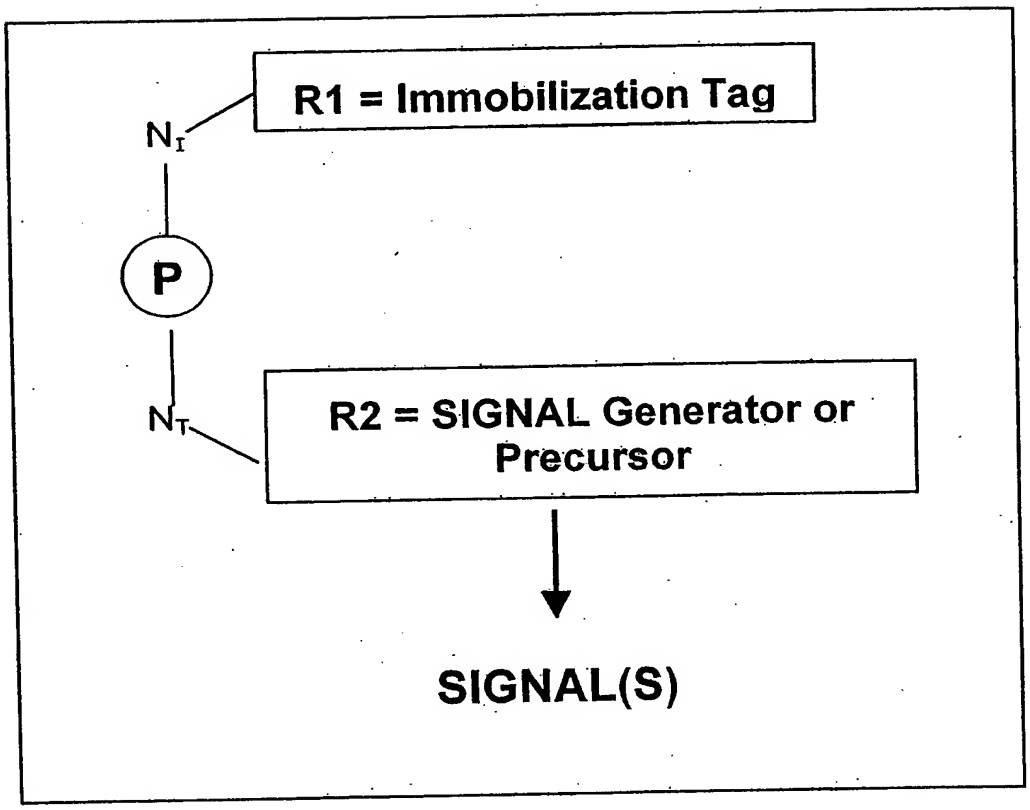

R1 = Immobilization Tag
N_I
P
N_T
R2 = SIGNAL Generator or Precursor
SIGNAL(S)

FIGURE 8

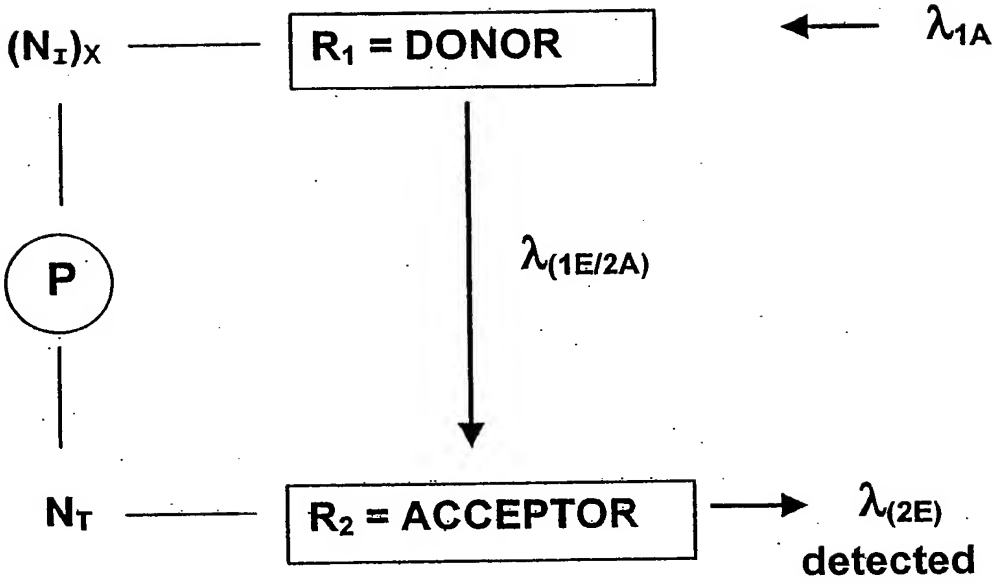

$(N_I)_X$
R_1 = DONOR
λ_{1A}
P
$\lambda_{(1E/2A)}$
N_T
R_2 = ACCEPTOR
$\lambda_{(2E)}$
detected

FIGURE 9

n $R_1N_1pN_2R_2$-OH

+

n $pppN_3R_3$

↓

n $R_1N_1pN_2R_2pN_3R_3$

where N_3 is a terminator

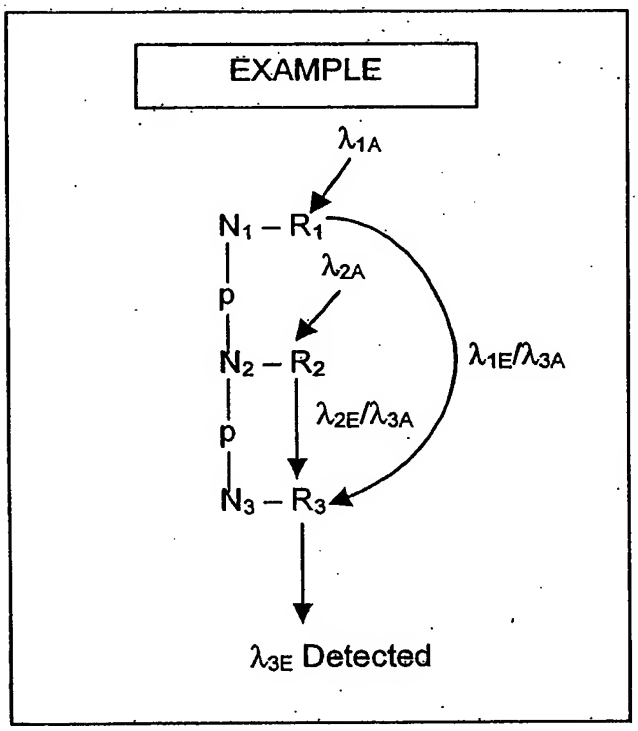


FIGURE 10

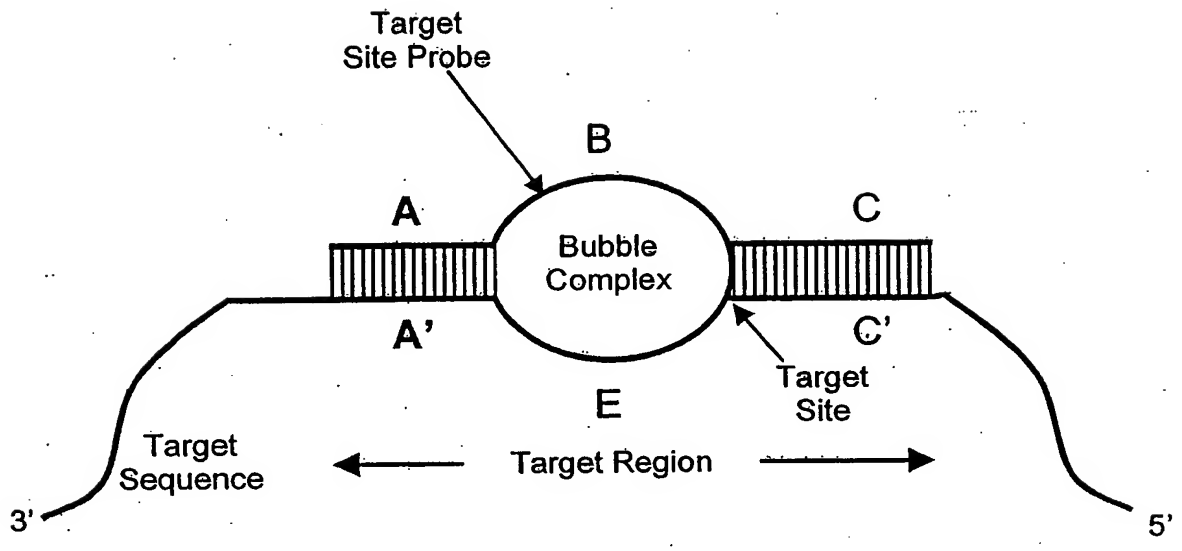

Target
Site Probe
B
A
C
Bubble
Complex
A'
C'
Target
Site
E
Target
Sequence
Target Region
3'
5'

FIGURE 11

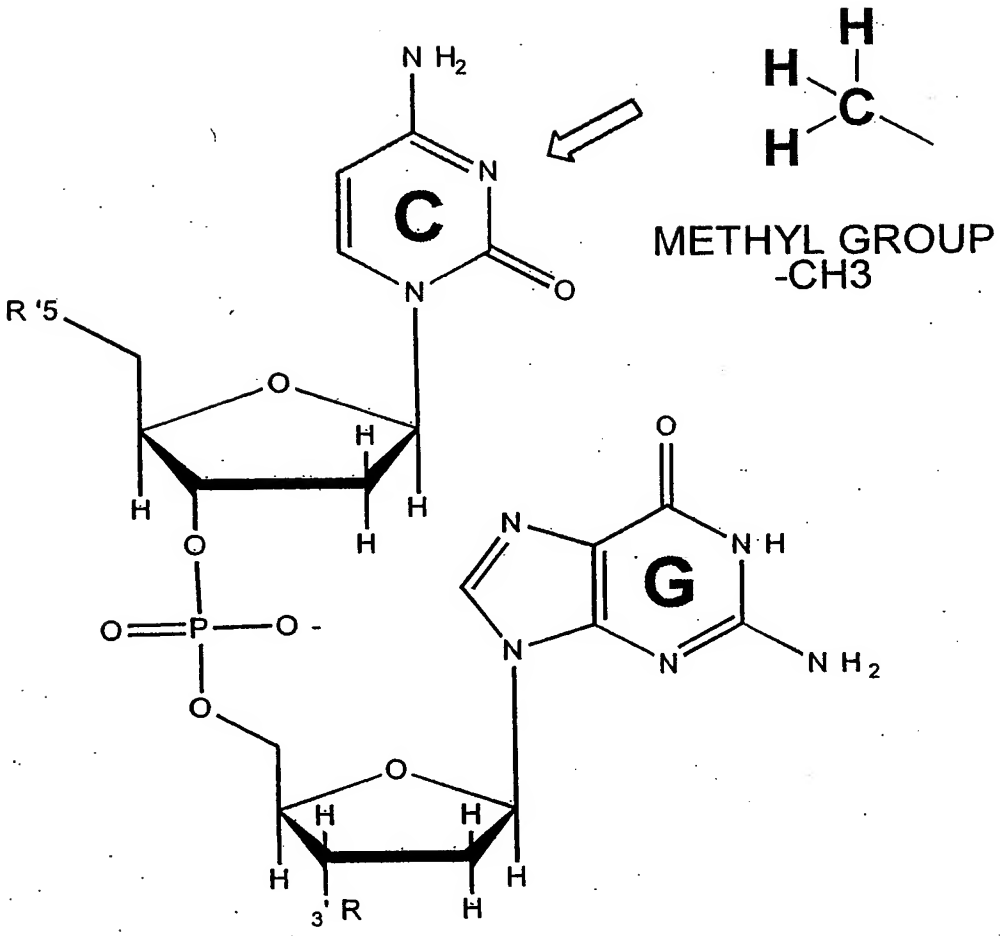


FIGURE 12

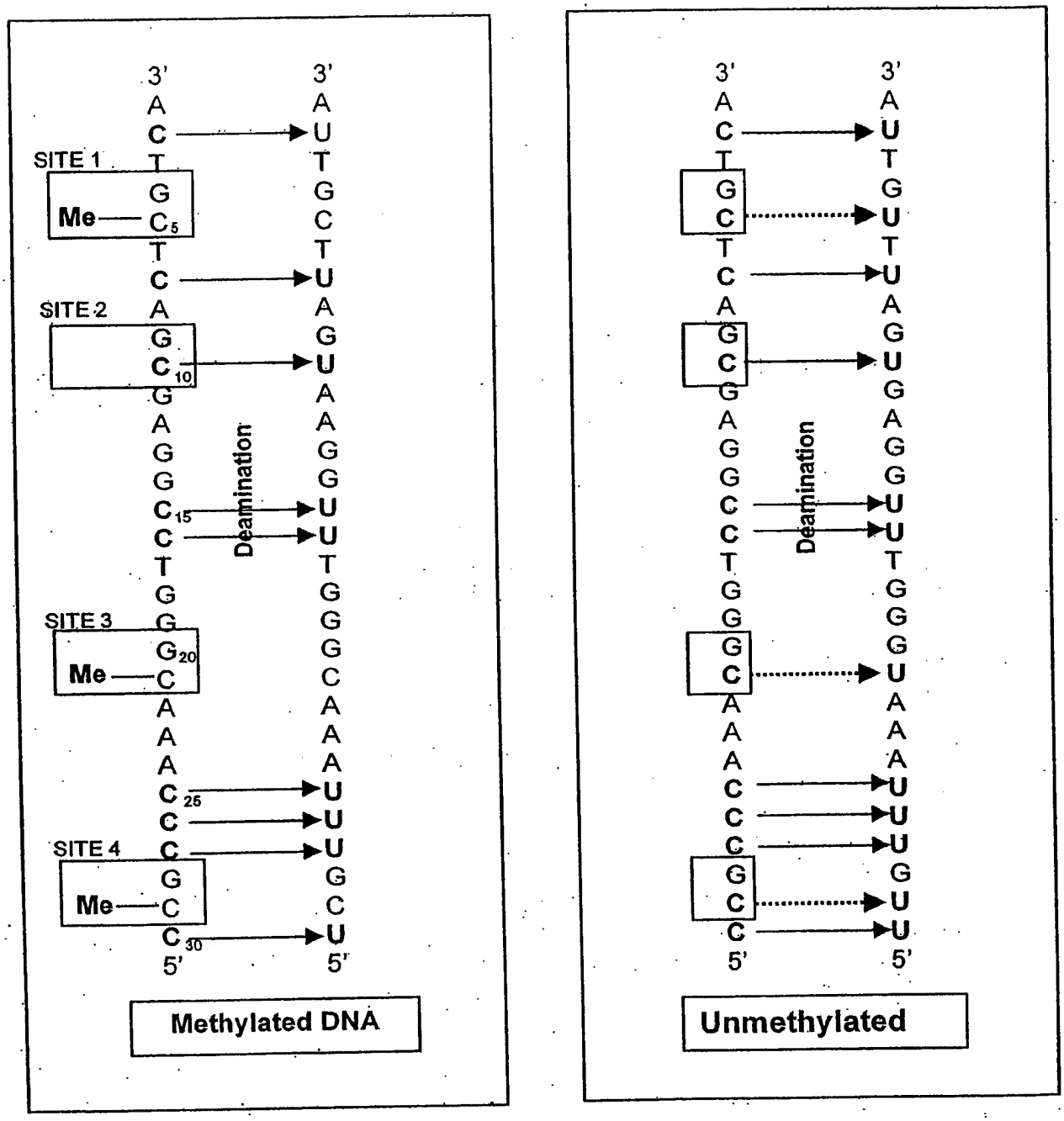

SITE 1
Me
SITE 2
SITE 3
Me
SITE 4
Me
Deamination
Methylated DNA
Deamination
Unmethylated

FIGURE 13

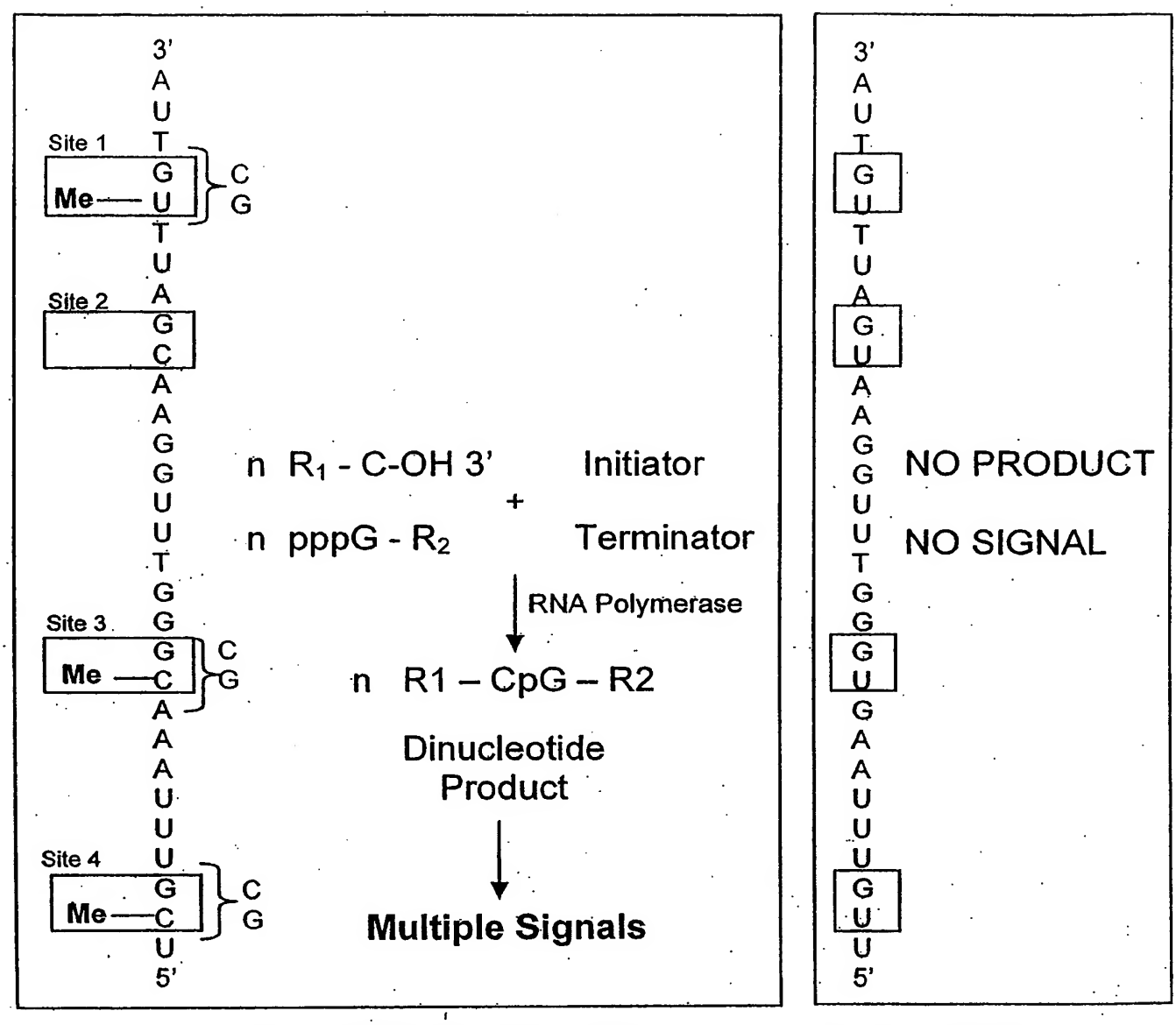

3'
A
U
T
Site 1
G
Me
U
C
G
T
U
A
Site 2
G
C
A
A
G
G
U
U
T
G
G
Site 3
G
Me
C
C
G
A
A
A
U
U
U
Site 4
G
Me
C
C
G
U
5'
n R1 - C-OH 3'
Initiator
+
n pppG - R2
Terminator
RNA Polymerase
n R1 – CpG – R2
Dinucleotide Product
Multiple Signals
3'
A
U
T
G
U
T
U
A
G
U
A
A
G
G
U
U
T
G
G
G
U
G
A
A
U
U
U
G
U
U
5'
NO PRODUCT
NO SIGNAL
Deaminated Methylated DNA
Deaminated Unmethylated DNA

FIGURE 14

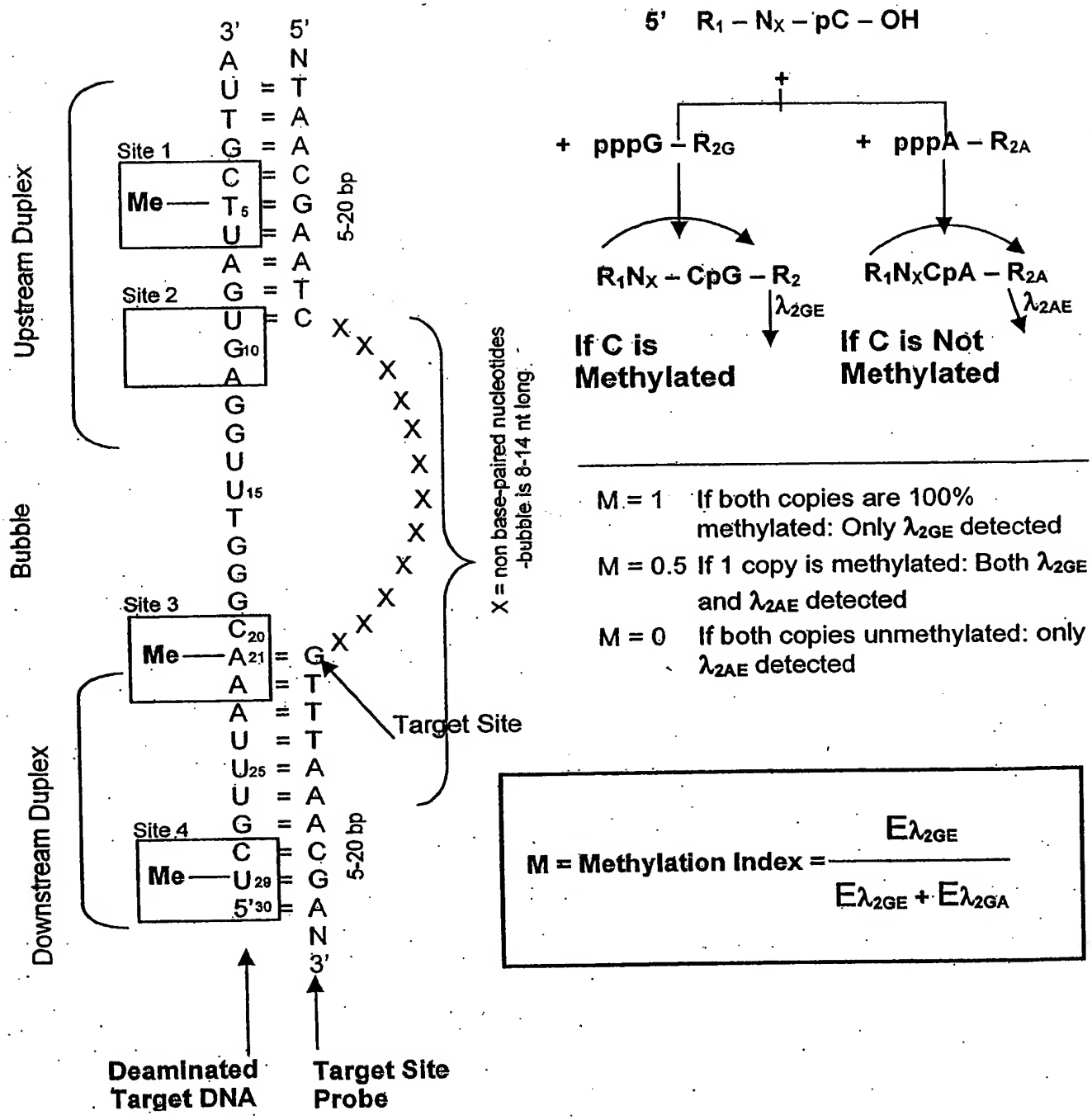

3' 5'
A N
U = T
T = A
G = A
C = C
Me — T5 = G
U = A
A = A
G = T
U = C
G10
A
G
G
U
U15
T
G
G
G
C20
Me — A21 = G
A = T
A = T
U = T
U25 = A
U = A
G = A
C = C
Me — U29 = G
5'30 = A
N
3'
Site 1
Site 2
Site 3
Site 4
5-20 bp
5-20 bp
Upstream Duplex
Bubble
Downstream Duplex
X = non base-paired nucleotides
-bubble is 8-14 nt long
Target Site
Deaminated Target DNA
Target Site Probe
5' R1 – NX – pC – OH
+
+ pppG – R2G
+ pppA – R2A
R1NX – CpG – R2
R1NXCpA – R2A
λ2GE
λ2AE
If C is Methylated
If C is Not Methylated
M = 1 If both copies are 100% methylated: Only λ2GE detected
M = 0.5 If 1 copy is methylated: Both λ2GE and λ2AE detected
M = 0 If both copies unmethylated: only λ2AE detected
M = Methylation Index = Eλ2GE / (Eλ2GE + Eλ2GA)

FIGURE 15

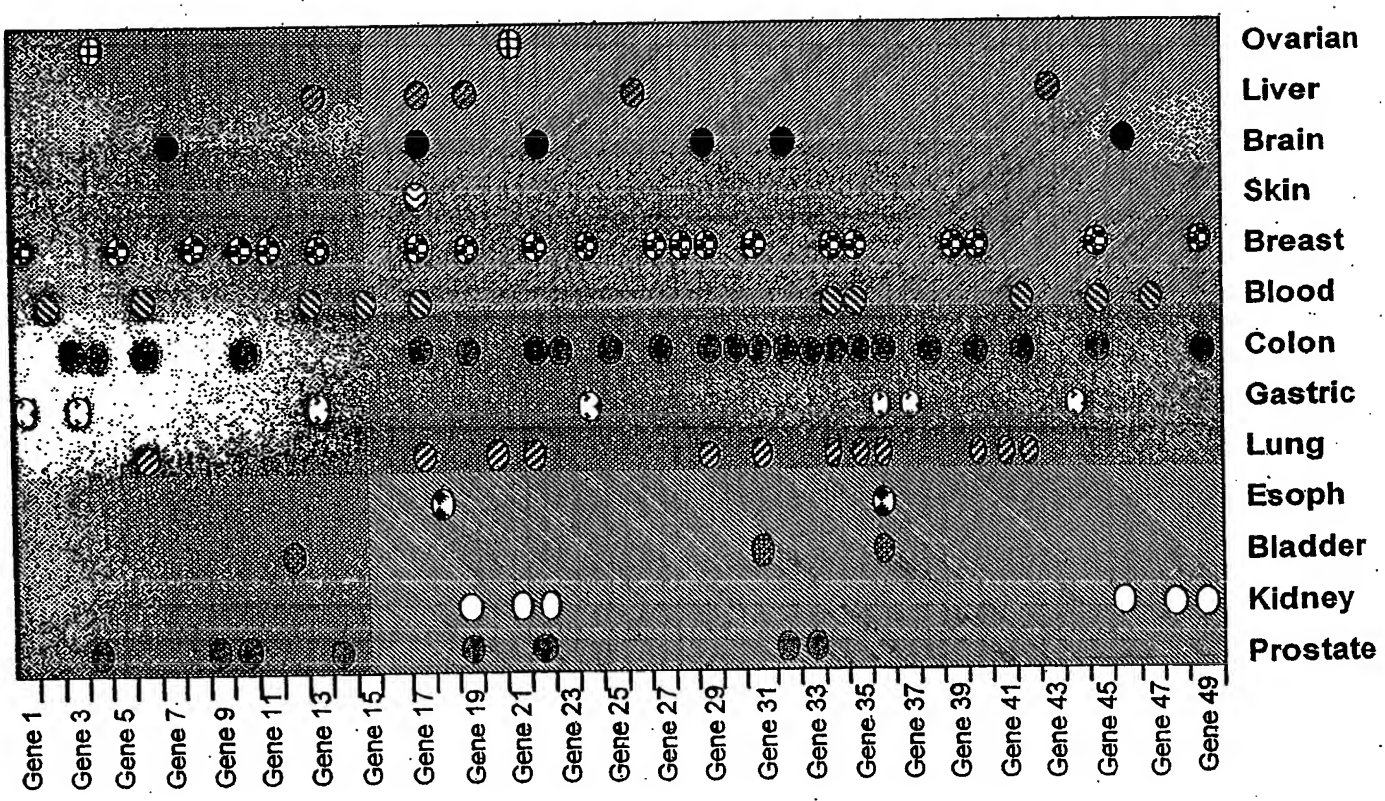

Ovarian
Liver
Brain
Skin
Breast
Blood
Colon
Gastric
Lung
Esoph
Bladder
Kidney
Prostate
Gene 1
Gene 3
Gene 5
Gene 7
Gene 9
Gene 11
Gene 13
Gene 15
Gene 17
Gene 19
Gene 21
Gene 23
Gene 25
Gene 27
Gene 29
Gene 31
Gene 33
Gene 35
Gene 37
Gene 39
Gene 41
Gene 43
Gene 45
Gene 47
Gene 49

FIGURE 16

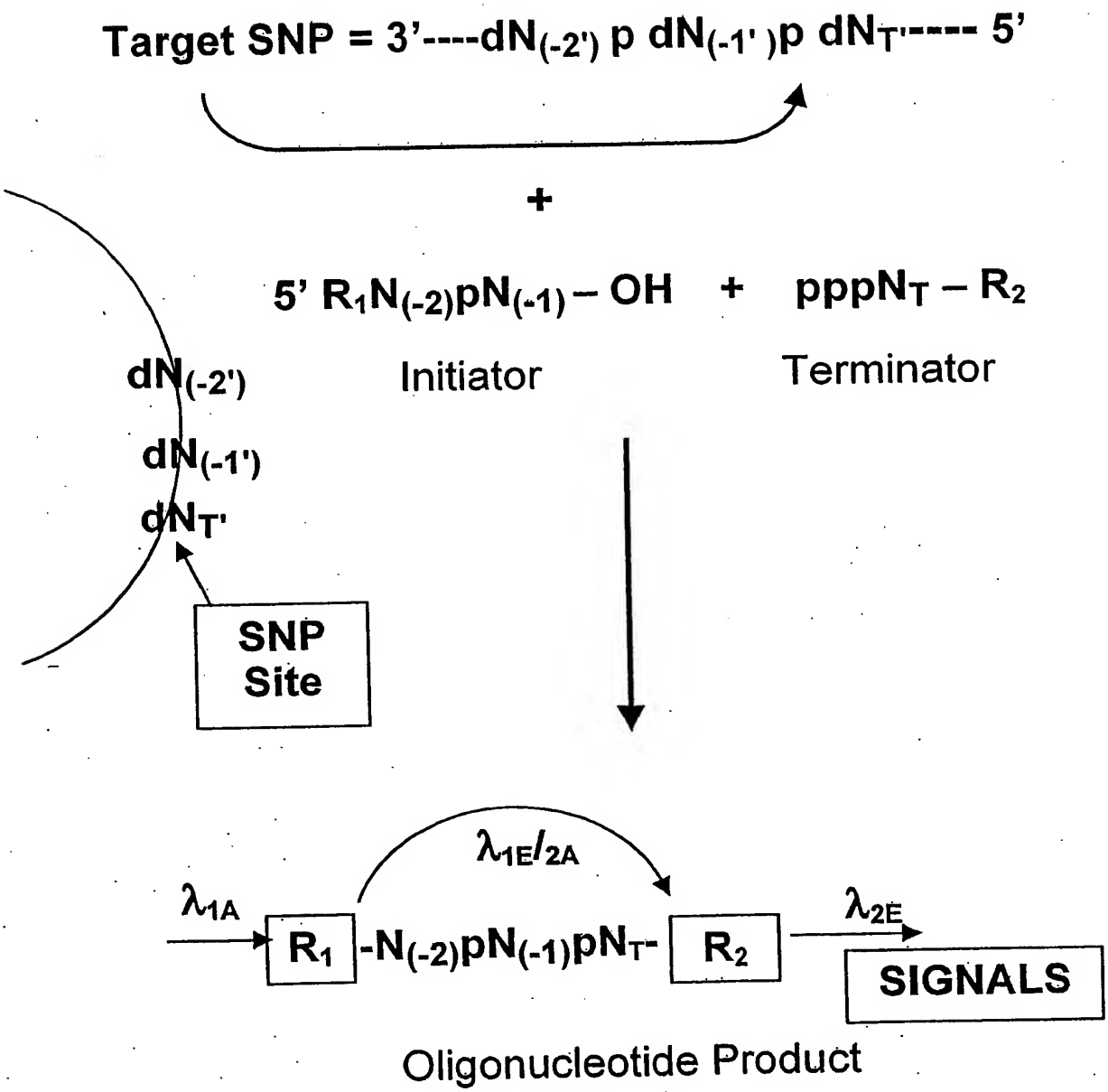

Target SNP = 3'----dN(-2') p dN(-1')p dNT'---- 5'
+
5' R1N(-2)pN(-1) – OH + pppNT – R2
Initiator
Terminator
dN(-2')
dN(-1')
dNT'
SNP Site
λ1E/2A
λ1A
R1
-N(-2)pN(-1)pNT-
R2
λ2E
SIGNALS
Oligonucleotide Product

FIGURE 17

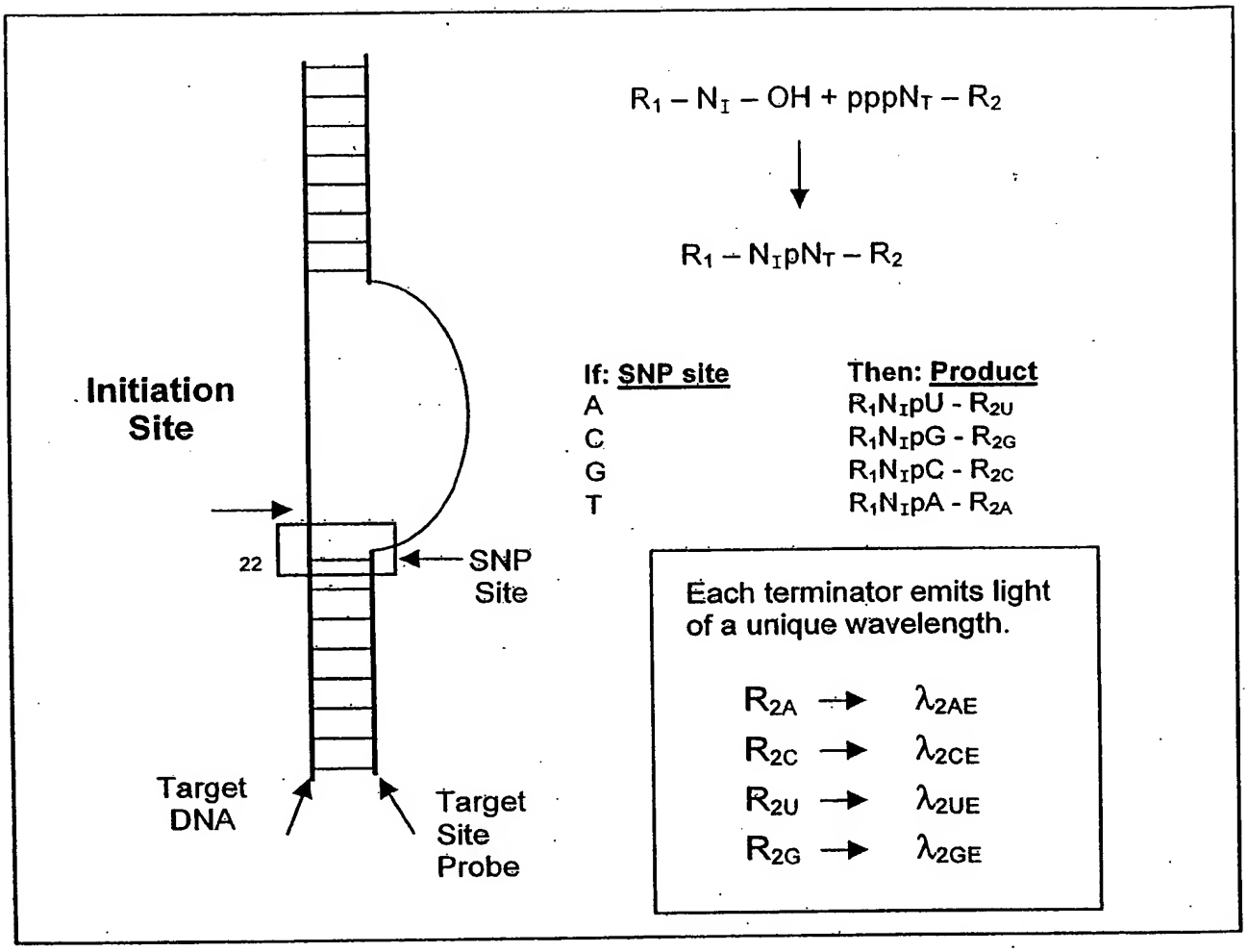

$R_1 - N_I - OH + pppN_T - R_2$
$R_1 - N_I pN_T - R_2$
Initiation Site
22
SNP Site
Target DNA
Target Site Probe
If: SNP site
A
C
G
T
Then: Product
$R_1N_IpU - R_{2U}$
$R_1N_IpG - R_{2G}$
$R_1N_IpC - R_{2C}$
$R_1N_IpA - R_{2A}$
Each terminator emits light of a unique wavelength.
$R_{2A} \rightarrow \lambda_{2AE}$
$R_{2C} \rightarrow \lambda_{2CE}$
$R_{2U} \rightarrow \lambda_{2UE}$
$R_{2G} \rightarrow \lambda_{2GE}$

FIGURE 18

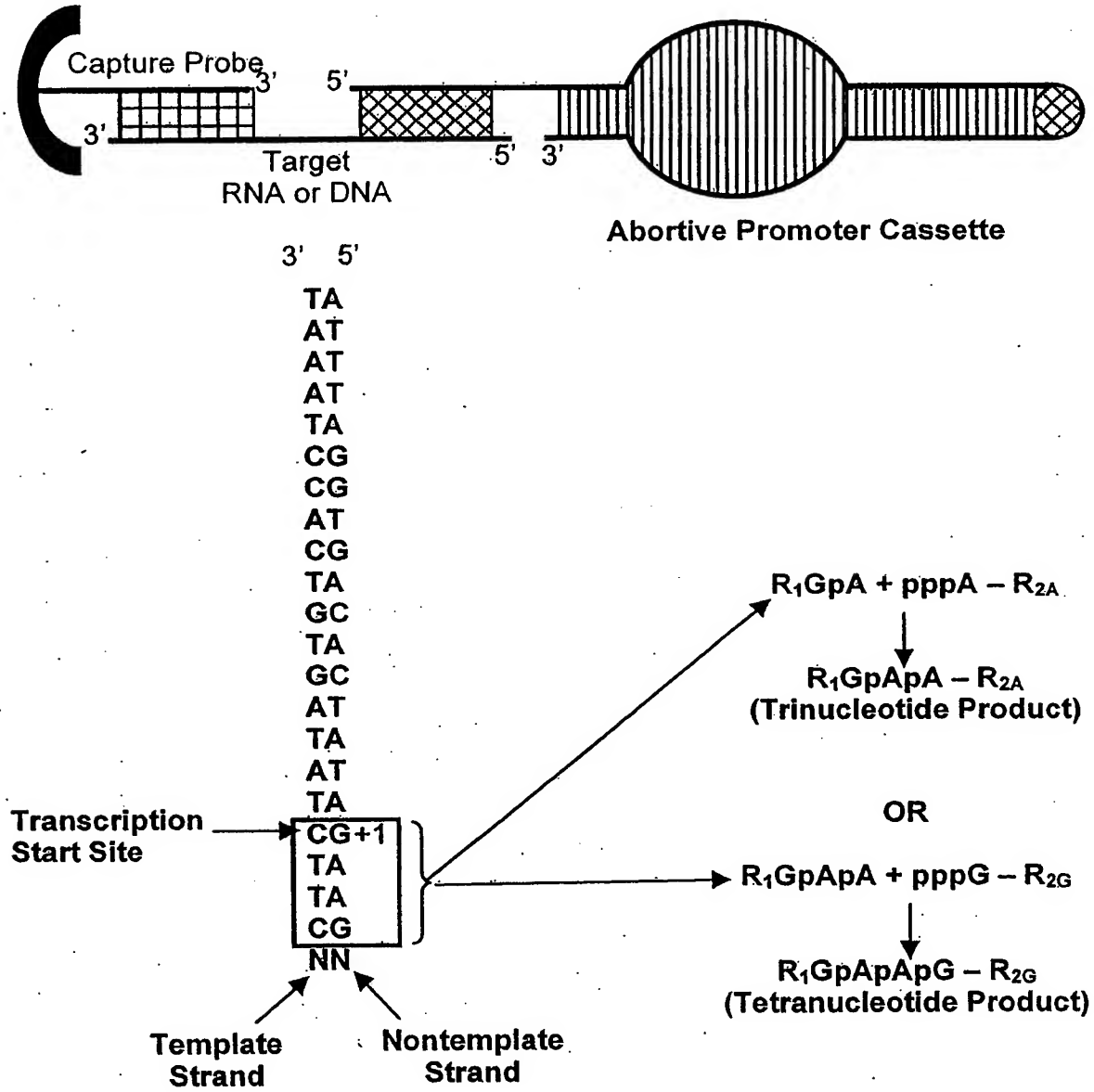

Capture Probe
3'
5'
3'
5'
3'
Target
RNA or DNA
Abortive Promoter Cassette
3' 5'
TA
AT
AT
AT
TA
CG
CG
AT
CG
TA
GC
TA
GC
AT
TA
AT
TA
Transcription
Start Site
CG+1
TA
TA
CG
NN
Template
Strand
Nontemplate
Strand
R1GpA + pppA – R2A
R1GpApA – R2A
(Trinucleotide Product)
OR
R1GpApA + pppG – R2G
R1GpApApG – R2G
(Tetranucleotide Product)

FIGURE 19

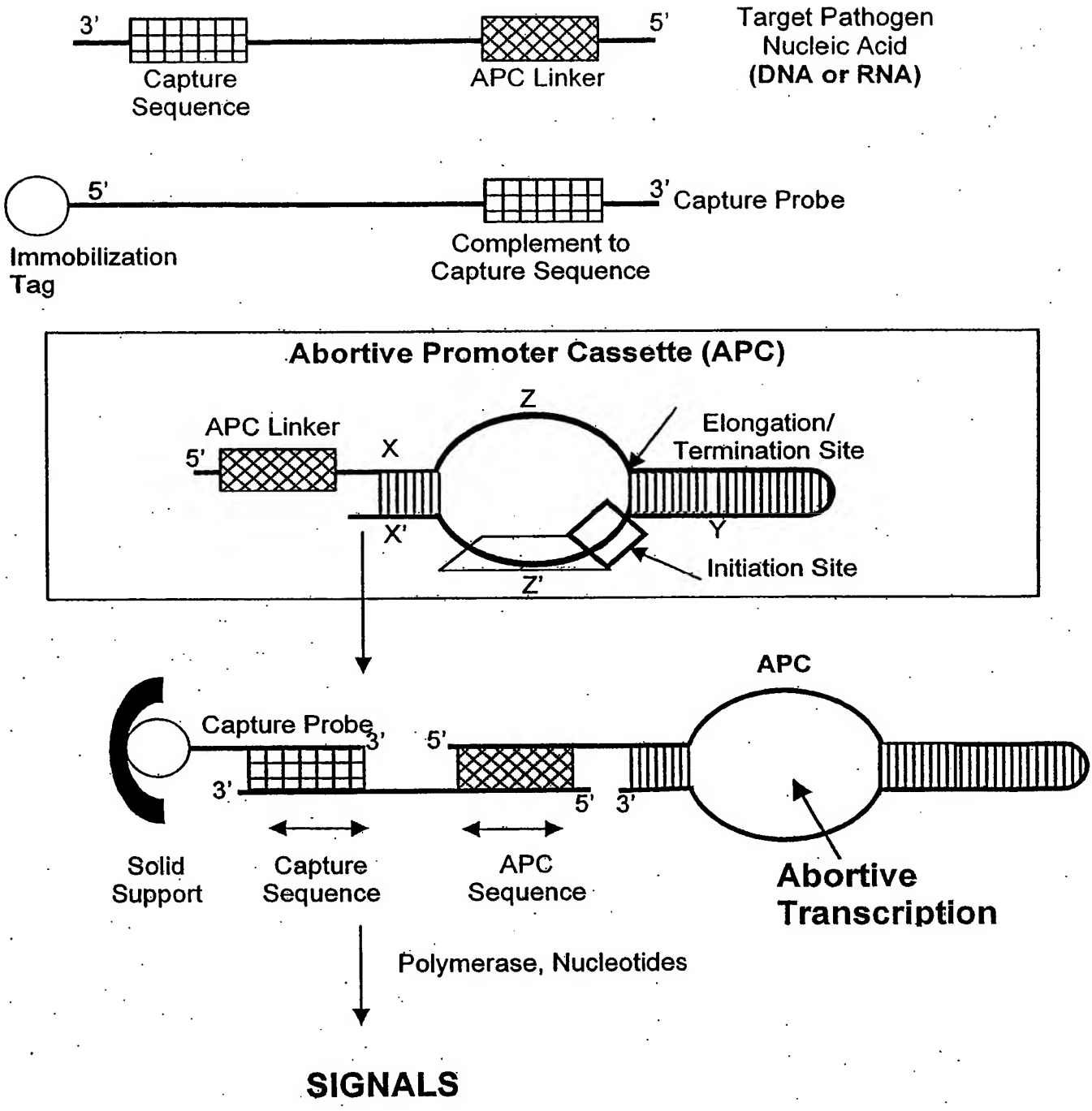

3'
5'
Capture Sequence
APC Linker
Target Pathogen Nucleic Acid (DNA or RNA)
5'
3'
Capture Probe
Immobilization Tag
Complement to Capture Sequence
Abortive Promoter Cassette (APC)
APC Linker
5'
X
Z
Elongation/ Termination Site
X'
Y
Initiation Site
Z'
APC
Capture Probe
3'
5'
3'
5'
3'
Solid Support
Capture Sequence
APC Sequence
Abortive Transcription
Polymerase, Nucleotides
SIGNALS

FIGURE 20

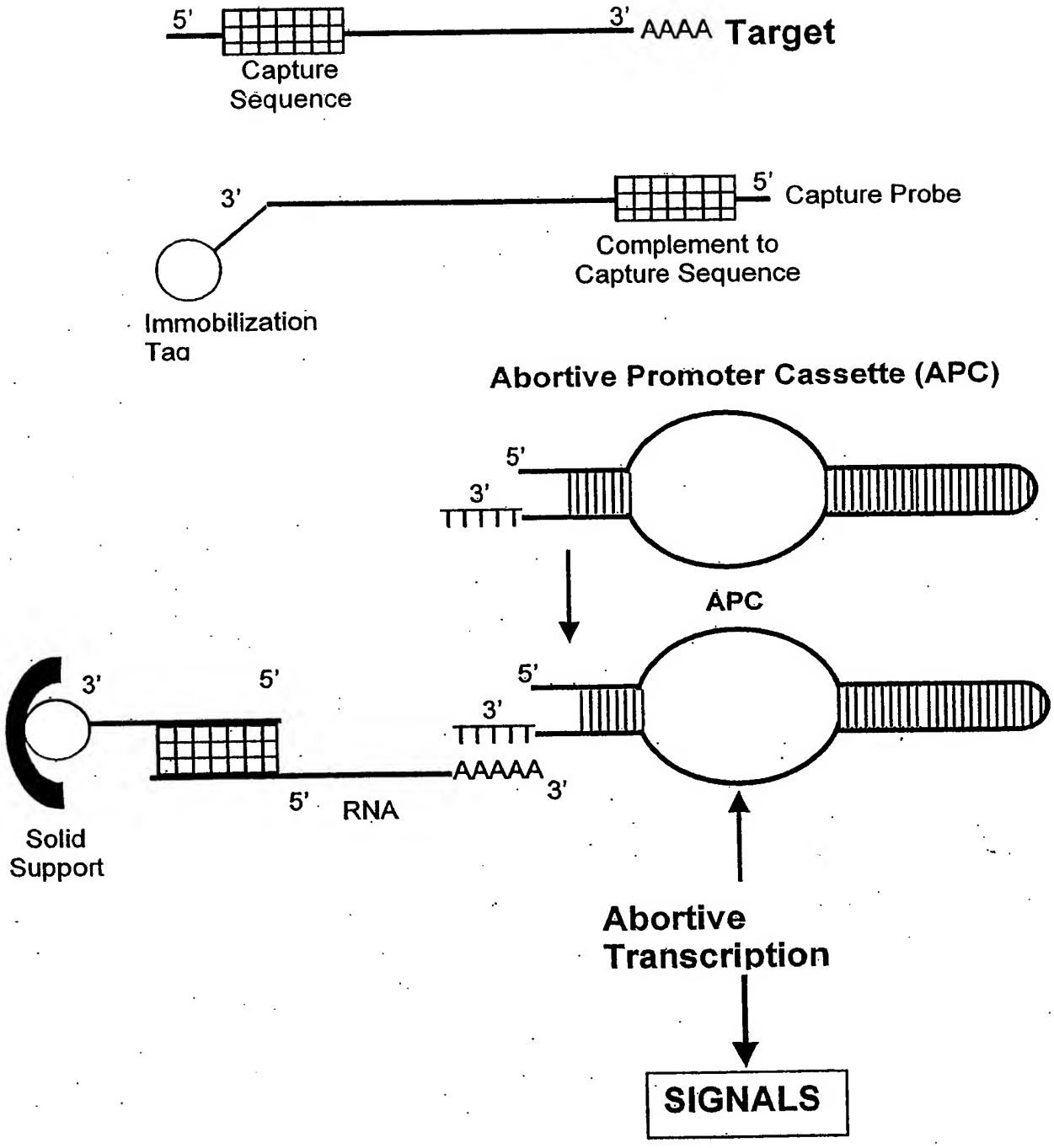

5'
3'
AAAA
Target
Capture
Sequence
3'
5'
Capture Probe
Complement to
Capture Sequence
Immobilization
Tag
Abortive Promoter Cassette (APC)
5'
3'
APC
3'
5'
5'
3'
AAAAA
3'
5'
RNA
Solid
Support
Abortive
Transcription
SIGNALS

FIGURE 21

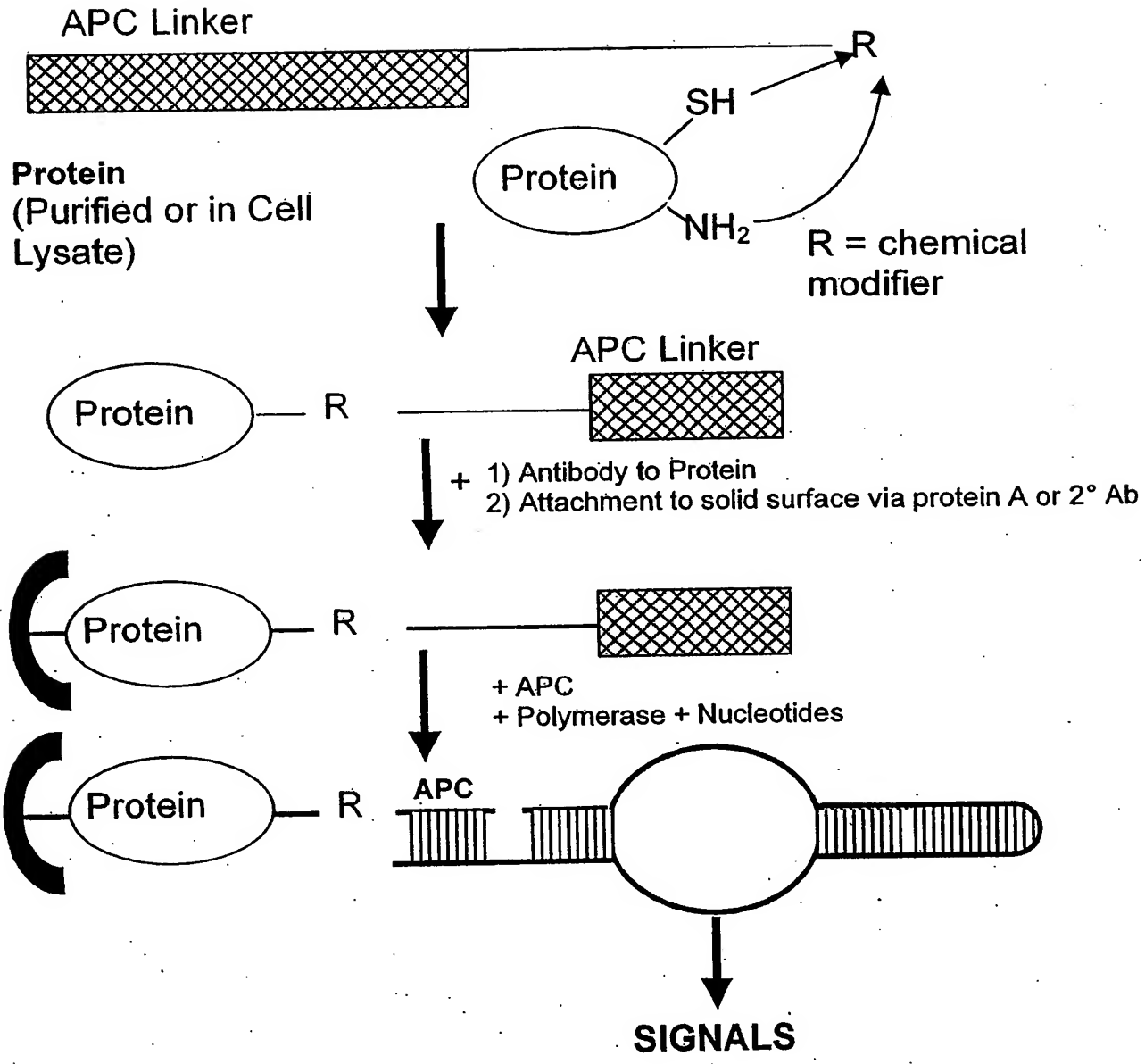

APC Linker
R
SH
Protein
NH2
Protein
(Purified or in Cell
Lysate)
R = chemical
modifier
APC Linker
Protein
R
+ 1) Antibody to Protein
2) Attachment to solid surface via protein A or 2° Ab
Protein
R
+ APC
+ Polymerase + Nucleotides
Protein
R
APC
SIGNALS

FIGURE 22

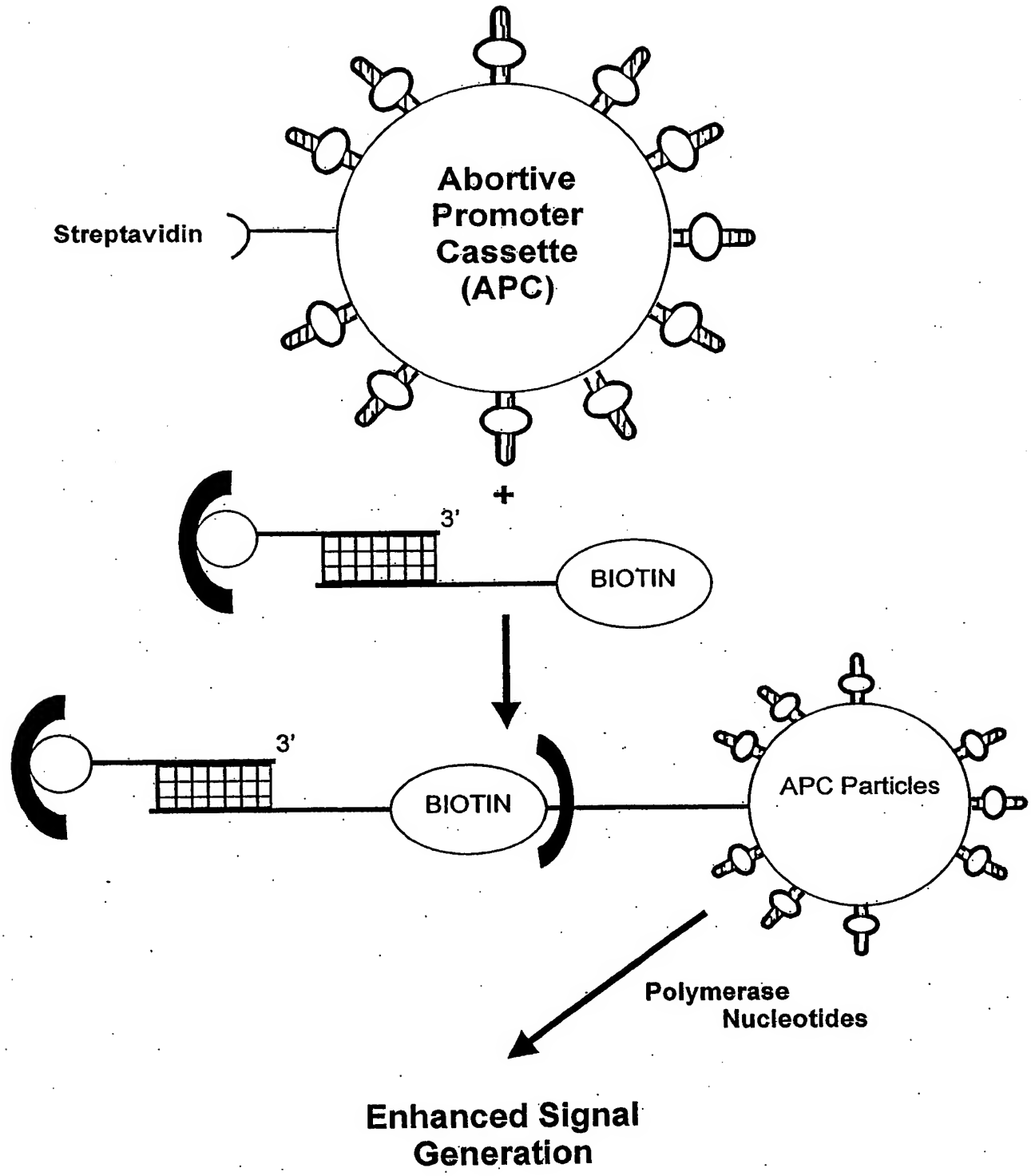

Abortive
Promoter
Cassette
(APC)
Streptavidin
+
3'
BIOTIN
3'
BIOTIN
APC Particles
Polymerase
Nucleotides
Enhanced Signal
Generation

FIGURE 23

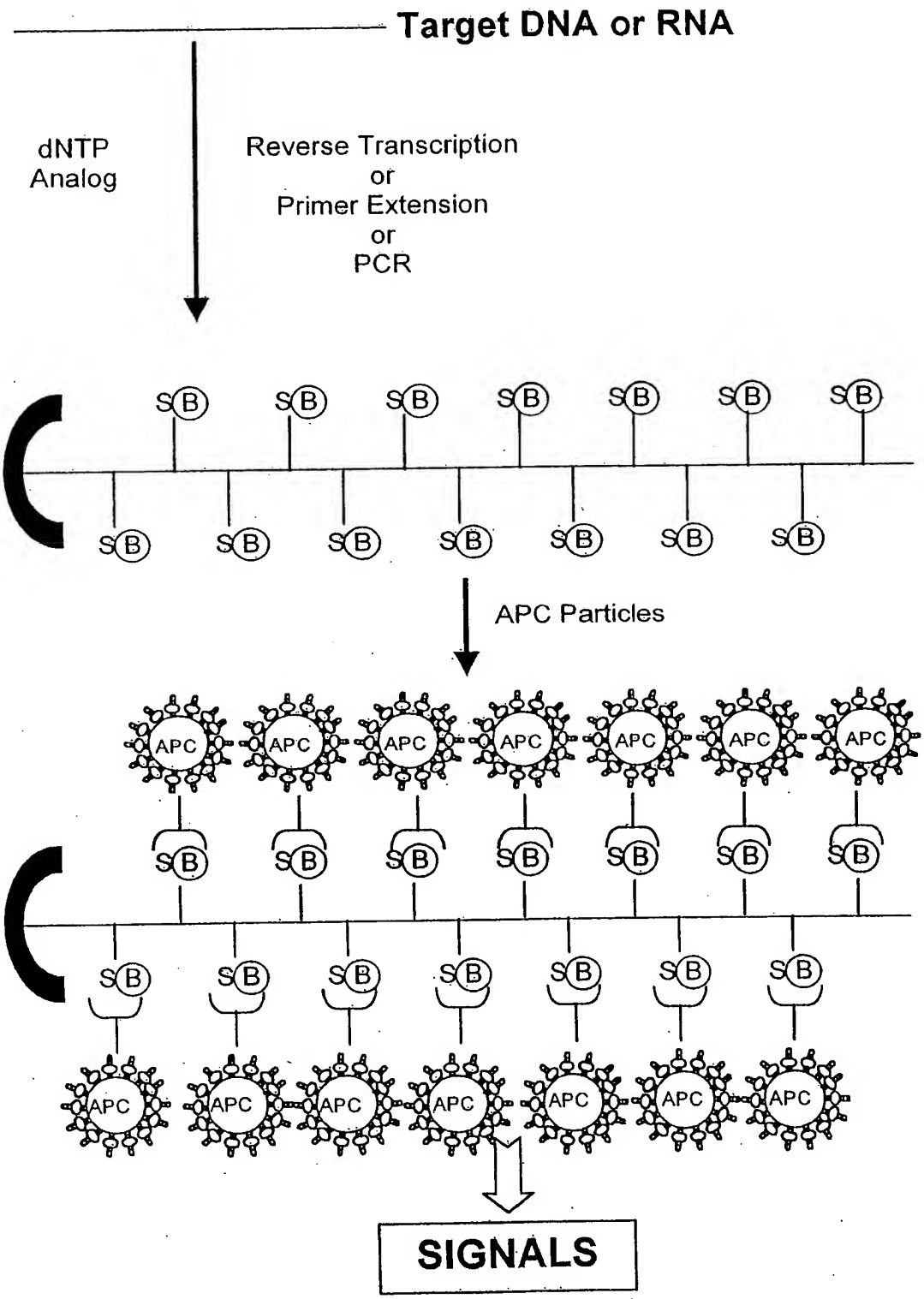

Target DNA or RNA
dNTP
Analog
Reverse Transcription
or
Primer Extension
or
PCR
S B
APC Particles
APC
SIGNALS

FIGURE 24

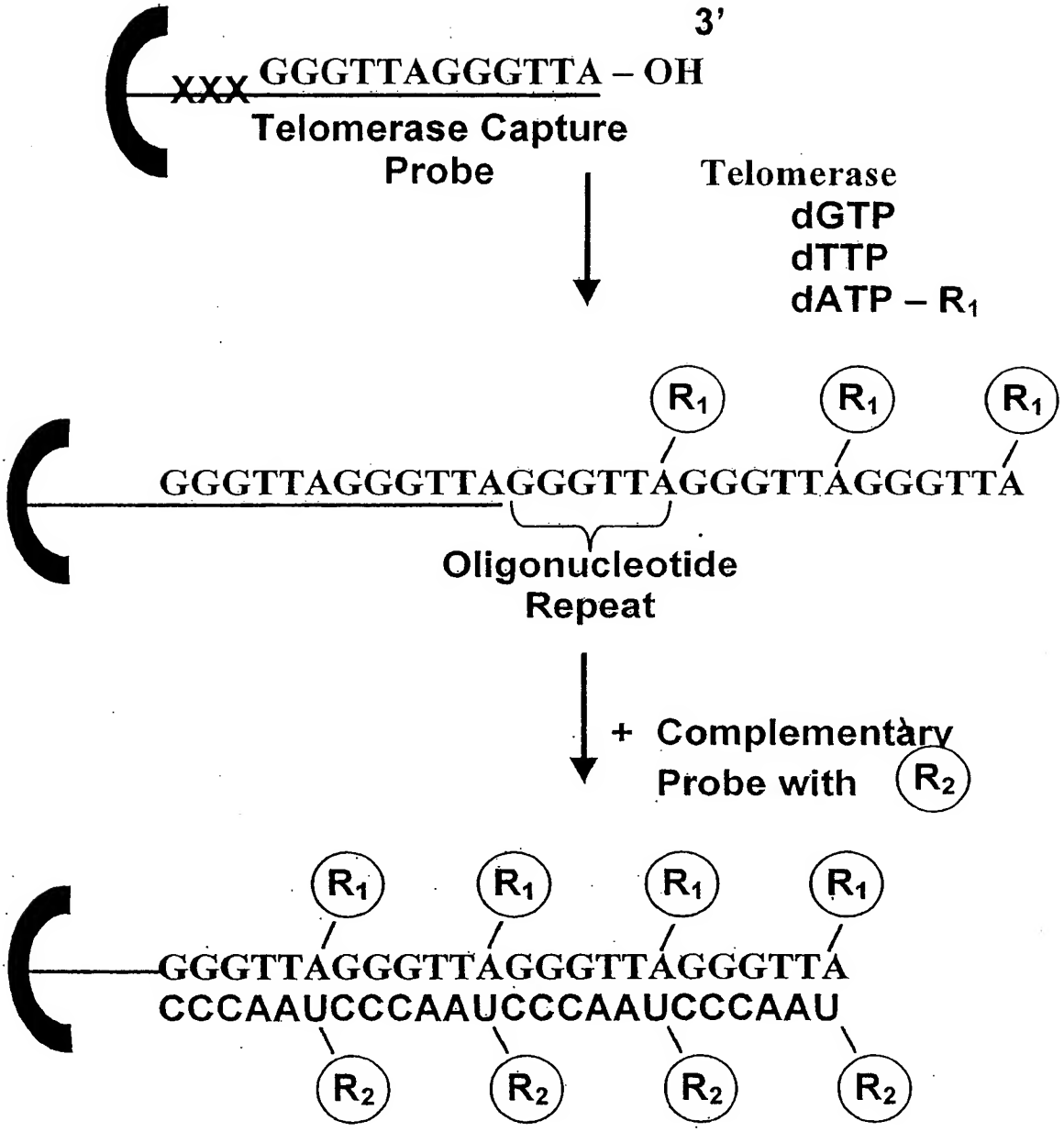

3'
XXX GGGTTAGGGTTA – OH
Telomerase Capture Probe
Telomerase
dGTP
dTTP
dATP – R1
R1
R1
R1
GGGTTAGGGTTAGGGTTAGGGTTAGGGTTA
Oligonucleotide Repeat
+ Complementary Probe with R2
R1
R1
R1
R1
GGGTTAGGGTTAGGGTTAGGGTTA
CCCAAUCCCAAUCCCAAUCCCAAU
R2
R2
R2
R2

FIGURE 25

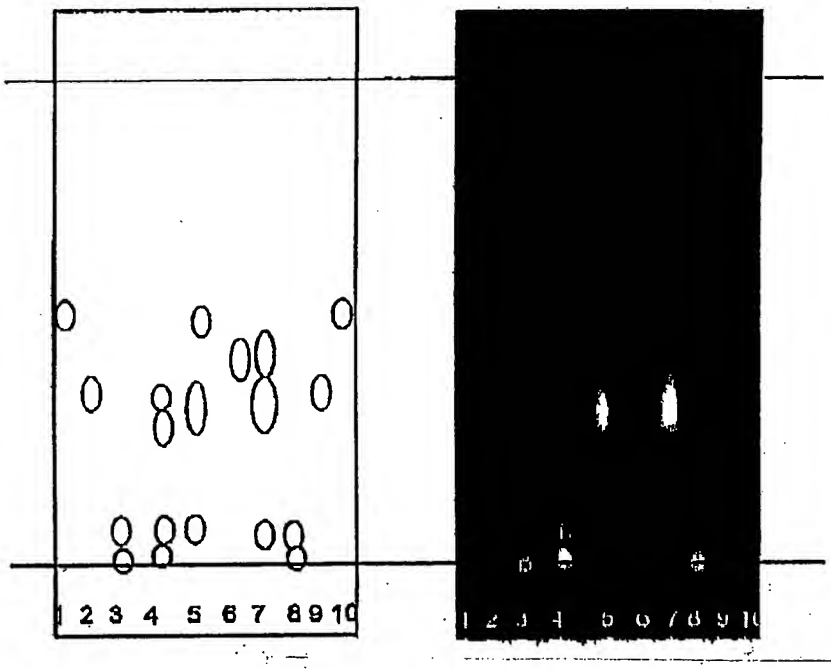


FIGURE 26

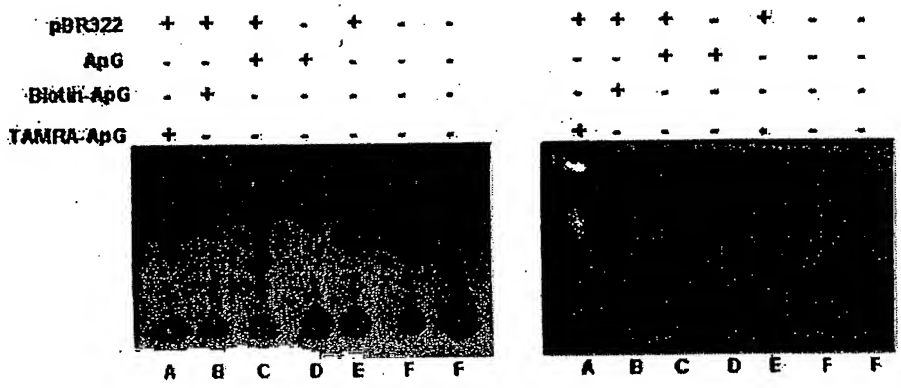


FIGURE 27

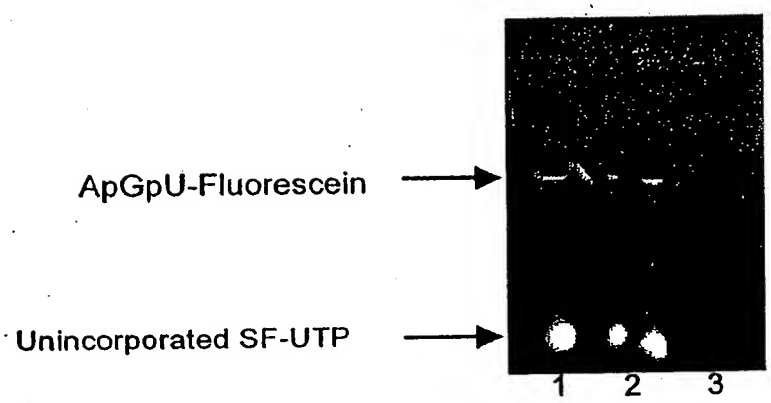


FIGURE 28

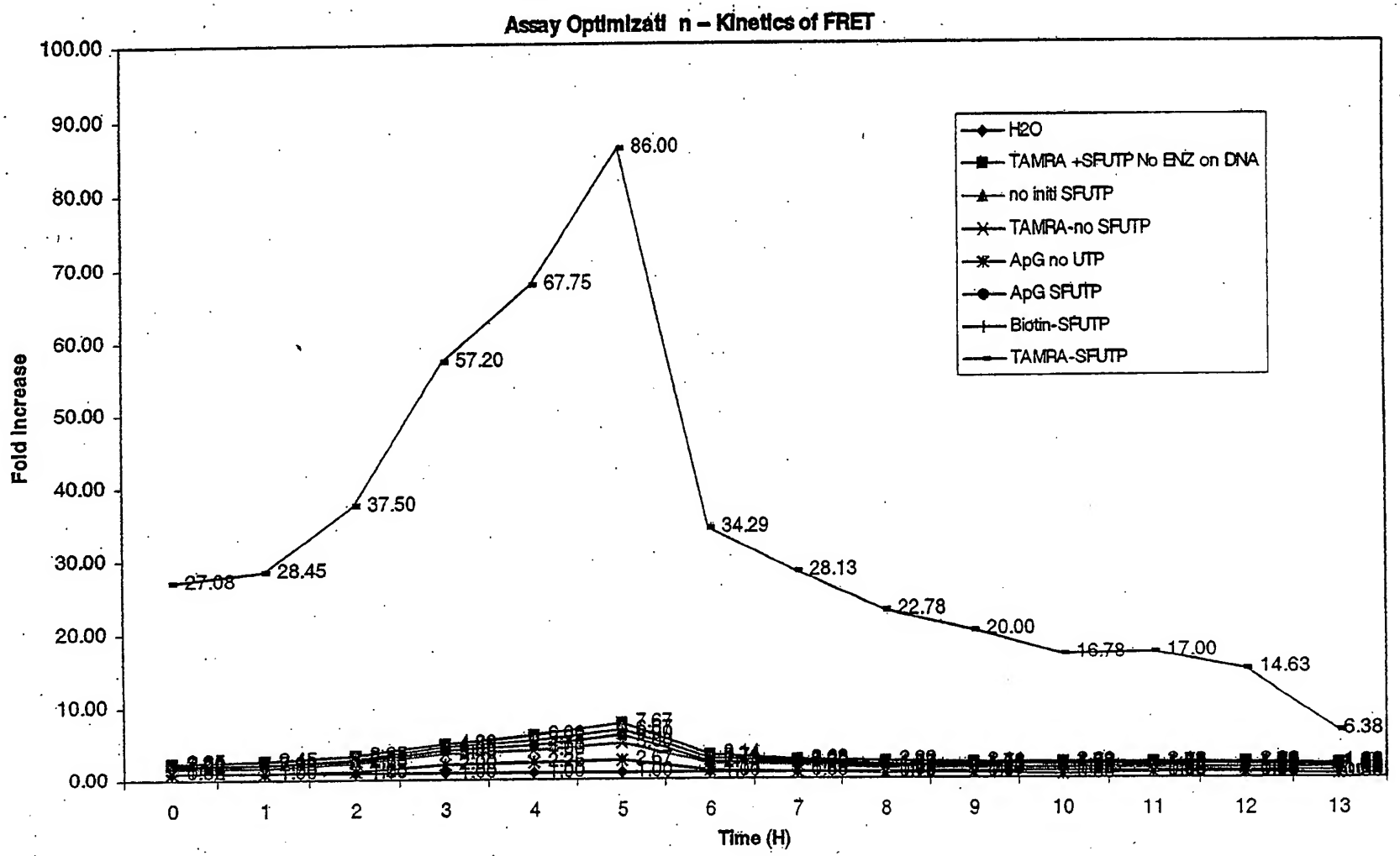

Assay Optimizati n – Kinetics of FRET
Fold Increase
Time (H)
H2O
TAMRA +SFUTP No ENZ on DNA
no initi SFUTP
TAMRA-no SFUTP
ApG no UTP
ApG SFUTP
Biotin-SFUTP
TAMRA-SFUTP
27.08
28.45
37.50
57.20
67.75
86.00
34.29
28.13
22.78
20.00
16.78
17.00
14.63
6.38

FIGURE 29A

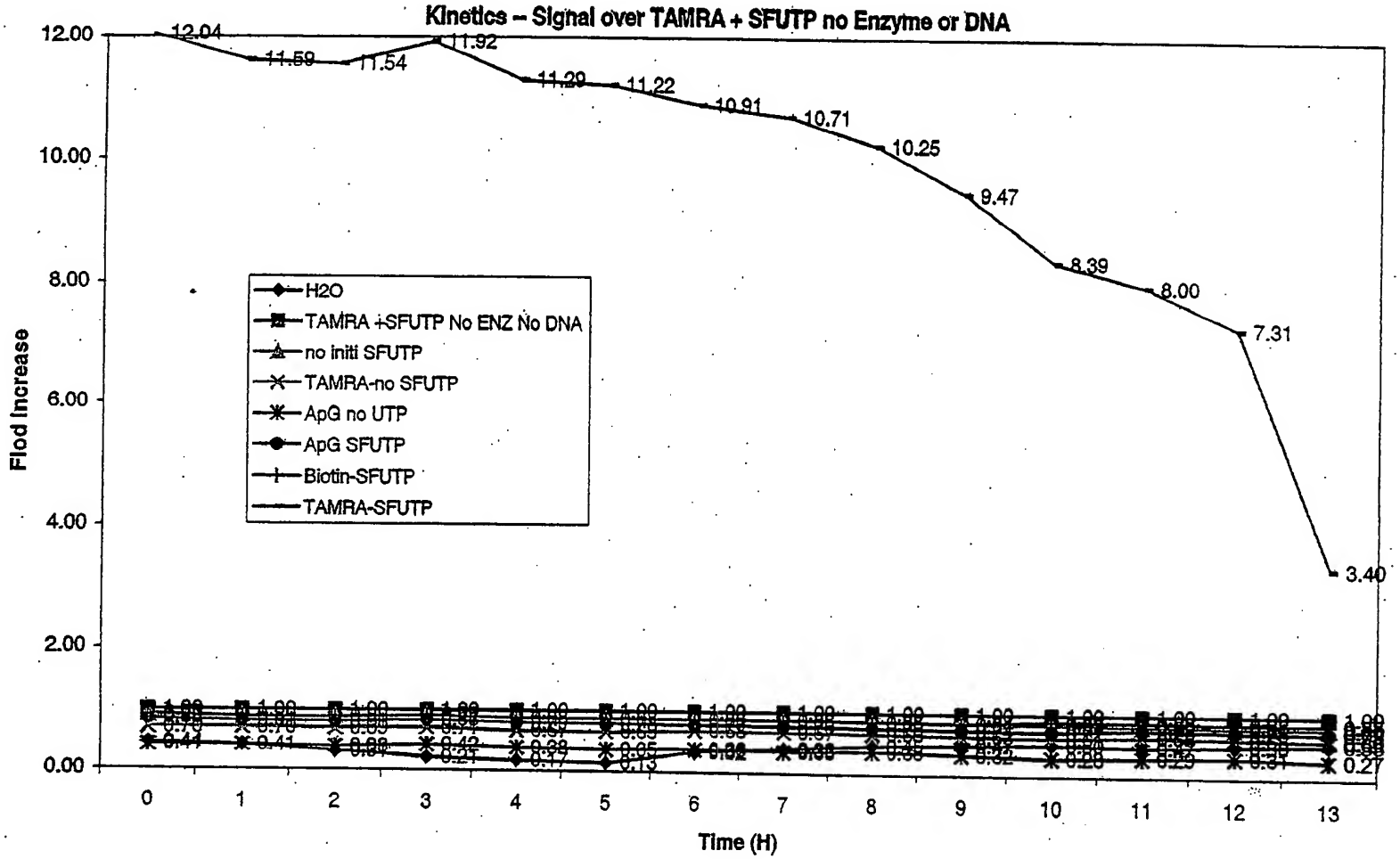

Kinetics -- Signal over TAMRA + SFUTP no Enzyme or DNA
Flod Increase
Time (H)
H2O
TAMRA +SFUTP No ENZ No DNA
no initi SFUTP
TAMRA-no SFUTP
ApG no UTP
ApG SFUTP
Biotin-SFUTP
TAMRA-SFUTP
12.04
11.59
11.54
11.92
11.29
11.22
10.91
10.71
10.25
9.47
8.39
8.00
7.31
3.40
0.27

FIGURE 29B

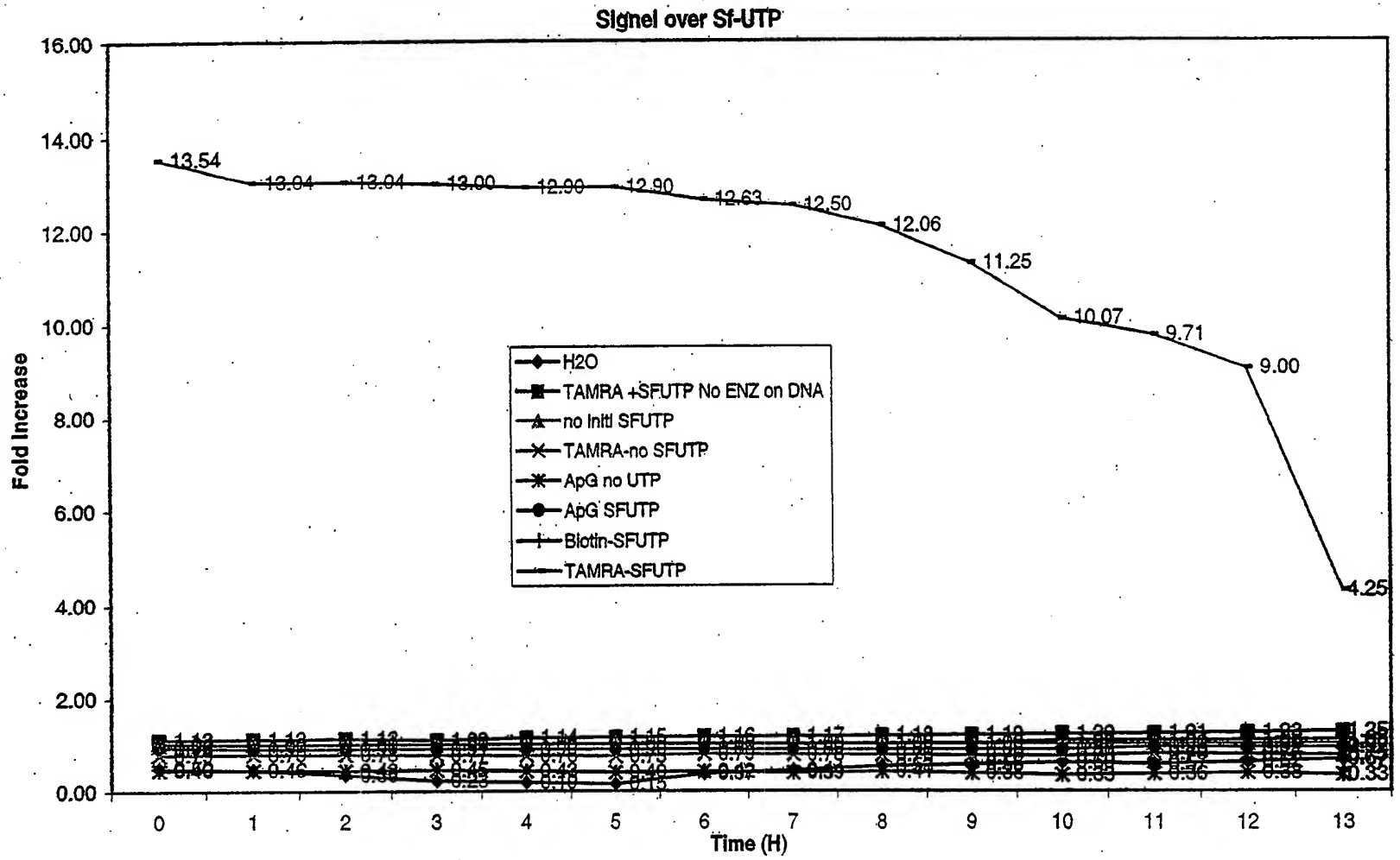

Signel over Sf-UTP
Fold Increase
Time (H)
H2O
TAMRA +SFUTP No ENZ on DNA
no Initl SFUTP
TAMRA-no SFUTP
ApG no UTP
ApG SFUTP
Biotin-SFUTP
TAMRA-SFUTP
13.54
13.04
13.04
13.00
12.90
12.90
12.63
12.50
12.06
11.25
10.07
9.71
9.00
4.25
0.33

FIGURE 29C

[redacted]ATTATCCAGT
AGGCAGATTAAGCATGTGCTTAAGGCATCAGCAAAGTCTGAGCAATCCATTTTTTAAAACGTAGTACATGTTTT
TGATAAGCTTAAAAAGTAGTAGTCACAGGAAAAATTAGAACTTTTACCTCCTTGCGCTTGTTATACTCTTTAGT
GCTGTTTAACTTTTCTTTGTAAGTGAGGGTGGTGGAGGGTGCCCATAATCTTTTCAGGGAGTAAGTTCTTCTT
GGTCTTTCTTTCTTTCTTTCTTTCTTTTTTTCTTGAGACCAAGTTTCGCTCTTGTCTCCCAGGCTGGAGTGCAA
TGGCGCGATCTCGGCTCACTGCAACCTCCGCCTTCTCCTGGGTTCAAGCGATTCTCCTACATCAGCCTCCGA
GTAGCTGGGATTACAGGCATGCGCCACCAAGCCCCGCTAATTTTGTATTTTTTAGTAGAGACAGGGTTTCGC
CATGTTGGTCAGGCTTGTCTCGAACTCCTGGCCTCAGGTGATCCGCCTGTCTCGGCCTCCCAGAATGCTGG
GATTATAGACGTGAGCCACCGCATCCGGACTTTCCTTTTATGTAATAGTGATAATTCTATCCAAAGCATTTTTT
TTTTTTTTTGAGTCGGAGTCTCATTCTGTCACCCAGGCTGGAGGGTGGTGGCGCGATCTCGGCTTACTGCAA
CCTCTGCCTCCCGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGAATTACACACGTGCGCCA
CCATGGCCAGCTAATTTTTGTATTTTTAGTAGAGACGGGGTGTCACCATTTTGGCCAAGCTGGCCTCGAACTC
CTGACCTCAGGTGATCTGCCCGCCTCGGCTTCCCAAAGTGCTGGGATTACAGGTGTGAGCCACCGCGTCCT
GCTCCAAAGCATTTTCTTTCTATGCCTCAAAACAAGATTGCAAGCCAGTCCTCAAAGCGGATAATTCAAGAGC
TAACAGGTATTAGCTTAGGATGTGTGGCACTGTTCTTAAGGCTTATATGTATTAATACATCATTTAAACTCACA
ACAACCCCTATAAAGCAGGGGGCACTCATATTCCCTTCCCCCTTTATAATTACGAAAAATGCAAGGTATTTTC
AGTAGGAAAGAGAAATGTGAGAAGTGTGAAGGAGACAGGACAGTATTTGAAGCTGGTCTTTGGATCACTGTG
CAACTCTGCTTCTAGAACACTGAGCACTTTTTCTGGTCTAGGAATTATGACTTTGAGAATGGAGTCCGTCCTT
CCAATGACTCCCTCCCCATTTTCCTATCTGCCTACAGGCAGAATTCTCCCCCGTCCGTATTAAATAAACCTCA
TCTTTTCAGAGTCTGCTCTTATACCAGGCAATGTACACGTCTGAGAAACCCTTGCCCCAGACAGCCGTTTTAC
ACGCAGGAGGGGAAGGGGAGGGGAAGGAGAGAGCAGTCCGACTCTCCAAAAGGAATCCTTTGAACTAGGG
TTTCTGACTTAGTGAACCCCGCGCTCCTGAAAATCAAGGGTTGAGGGGGTAGGGGGACACTTTCTAGTCGTA
CAGGTGATTTCGATTCTCGGTGGGGCTCTCACAACTAGGAAAGAATAGTTTTGCTTTTTCTTATGATTAAAAGA
AGAAGCCATACTTTCCCTATGACACCAAACACCCCGATTCAATTTGGCAGTTAGGAAGGTTGTATCGCGGAG
GAAGGAAACGGGGCGGGGGCGGATTTCTTTTTAACAGAGTGAACGCACTCAAACACGCCTTTGCTGGCAGG
CGGGGGAGCGCGGCTGGGAGCAGGGAGGCCGGAGGGCGGTGTGGGGGGCAGGTGGGGAGGAGCCCAGT
CCTCCTTCCTTGCCAACGCTGGCTCTGGCGAGGGCTGCTTCCGGCTGGTGCCCCCGGGGGAGACCCAACC
TGGGGCGACTTCAGGGGTGCCACATTCGCTAAGTGCTCGGAGTTAATAGCACCTCCTCCGAGCACTCGCTC
ACGGCGTCCCCTTGCCTGGAAAGATACCGCGGTCCCTCCAGAGGATTTGAGGGACAGGGTCGGAGGGGGC
TCTTCCGCCAGCACCGGAGGAAGAAAGAGGAGGGGCTGGCTGGTCACCAGAGGGTGGGGCGGACCGCGT
GCGCTCGGCGGCTGCGGAGAGGGGGAGAGCAGGCAGCGGGCGGCGGGGAGCAGCATGGAGCCGGCGGC
GGGGAGCAGCATGGAGCCTTCGGCTGACTGGCTGGCCACGGCCGCGGCCCGGGGTCGGGTAGAGGAGGT
GCGGGCGCTGCTGGAGGCGGGGGCGCTGCCCAACGCACCGAATAGTTACGGTCGGAGGCCGATCCAGGT
GGGTAGAGGGTCTGCAGCGGGAGCAGGGGATGGCGGGCGACTCTGGAGGACGAAGTTTGCAGGGGAATT
GGAATCAGGTAGCGCTTCGATTCTCCGGAAAAAGGGGAGGCTTCCTGGGGAGTTTTCAGAAGGGGTTTGTA
ATCACAGACCTCCTCCTGGCGACGCCCTGGGGGCTTGGGAAGCCAAGGAAGAGGAATGAGGAGCCACGCG
CGTACAGATCTCTCGAATGCTGAGAAGATCTGAAGGGGGGAACATATTTGTATTAGATGGAAGTATGCTCTTT
ATCAGATACAAAATTTACGAACGTTTGGGATAAAAAGGGAGTCTTAAAGAAATGTAAGATGTGCTGGGACTAC
TTAGCCTCCAATTCACAGATACCTGGATGGAGCTTATCTTTCTTACTAGGAGGGATTATCAGTGGAAATCTGT
GGTGTATGTTGGAATAAATATCGAATATAAATTTTGATCGAAATTATTCAGAAGCGGCCGGGCGCGGTGCCTC
ACGCCTTGTAATCCCTTCACTTTGGGAGATCAAGGCGGGGGGGAATCACCTGAGGTCGGGAGTTCGAGACCA
GCCTGGCCAACAGGTGAAACCTCGCCTCTACTAAAAATACAAAAAGTAGCCGGGGGTGGTGGCAGGCGCCT
GTAATCCCAGCTACTCGGGAGGTTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCTGAGGTTGTAGTGAAC
AGCGAGATGGAGCCACTTCACTCCAGCCTGGGTGACAGAGTGAGACTTTGTCGAAAGAAAGAAAGAGAGAA
AGAGAGAGAGAAAAATTATTCAGAAGCAACTACATATTGTGTTTATTTTTAACTGAGTAGGGCAAATAAATATA
TGTTTGCTGTAGGAACTTAGGAAATAATGAGCCACATTCATGTGATCATTCCAGAGGTAATATGTAGTTACCAT
TTTGGGAATATCTGCTAACATTTTTGCTCTTTTACTATCTTTAGCTTACTTGATATAGTTTATTTGTGATAAGAG
TTTTCAATTCCTCATTTTTGAACAGAGGTGTTTCTCCTCTCCCTACTCCTGTTTTGTGAGGGAGTTAGGGGAG
GATTTAAAAGTAATTAATACATGGGTAACTTAGCATCTCTAAAATTTTGCCAACAGCTTGAACCCGGGAGTTTG
GCTTTGTAGTCCTACAATATCTTAGAAGAGACCTTATTTGTTTAAAAACAAAAAGGAAAAAGAAAAGTGGATAG
TTTTGACAATTTTTAATGGAG

Figure 30

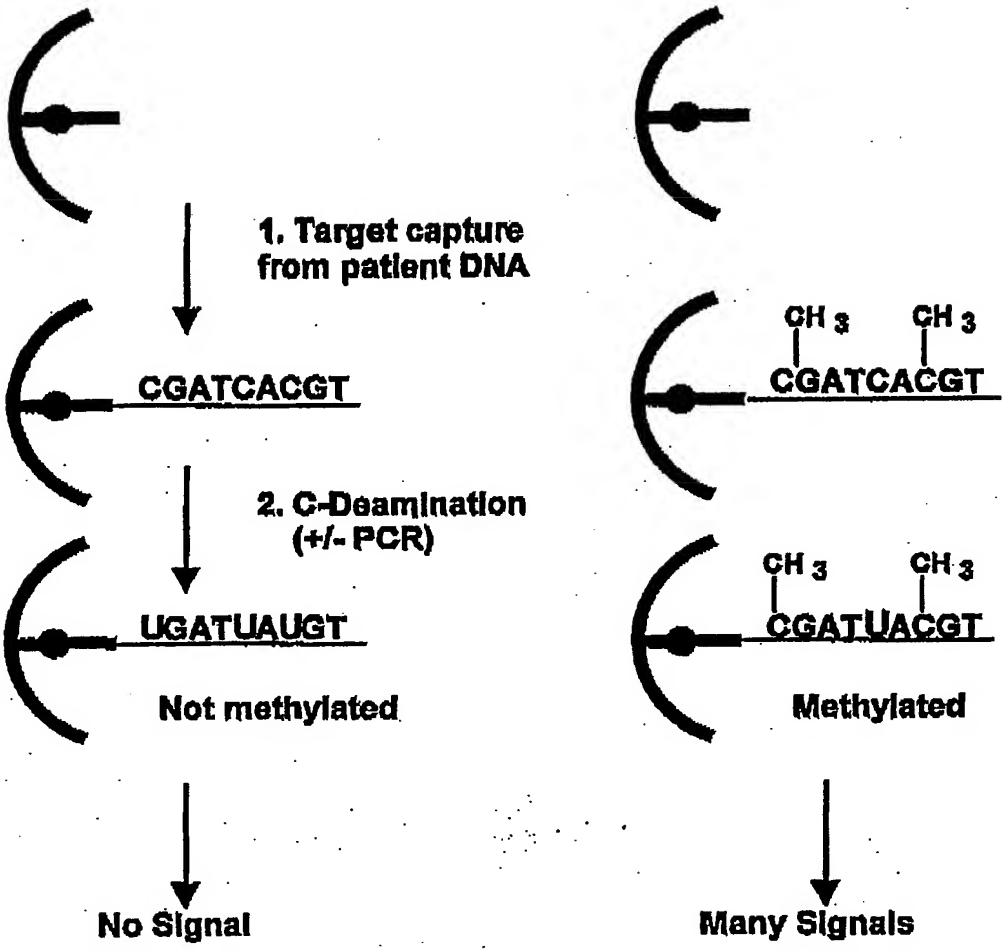

1. Target capture
from patient DNA
CGATCACGT
2. C-Deamination
(+/- PCR)
UGATUAUGT
Not methylated
No Signal
CH 3
CH 3
CGATCACGT
CH 3
CH 3
CGATUACGT
Methylated
Many Signals

FIGURE 31